analytica Vietnam

7th ANALYTICA VIETNAM CONFERENCE

VIỆN KIỂM NGHIỆM THUỐC THÀNH PHỐ HỒ CHÍ MINH
Institute of Drug Quality Control – HoChiMinh City, Ministry of Health

NEW LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR QUALITY CONTROL OF NITROSAMINE IMPURITIES (NDMA, NDEA, AND NMBA) IN SOME PHARMACEUTICAL PRODUCTS (VALSARTAN, IRBESARTAN, LOSARTAN, CANDESARTAN, AND TELMISARTAN TABLETS)

Ha Minh Hien, Phan Nguyen Truong Thang, Le Thanh Hoang, Tran Viet Hung*

12 th May, 2022

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4. IDQC HCMC Labs equipments, the future of multi-functional QC labs, quality control for food, pharmaceuticals, cosmetics, vaccine and bio-medical products, seeking for research co-operation, support and service from State bodies and private companies

1. Some biographies as HPLChromatography –User

Dr. Chromato "Graph Er"

- Graduated, pharmacist 1994, HUP, 13 Le Thanh Tong St, Hanoi, used to be an analyst in NIDQC, HPLChromatography –User, Merck – Hitachi 655 12A since 1995
- In 1995 June, trained in 17 – 19 Le Thanh Tong St, Faculty of Chemistry, Hanoi University (university of natural sciences), the first time met Prof. Pham Hung Viet
- In 1997 July – August., trained in Chromatography Development and Education Center, Prof. Nguyen Xuan Dung, (EDCVN, edcvn@hn.vnn.vn, Building C10B, University of Science and Technology (so call PolyTechnics Univerisity) No. 1 Dai Co Viet)
- 1996, 1998, NIDQC purchased 03 HP1100 (Hewlett Packard, changed to Agilent), Chemstation Softwere…HP AminoQuant (1998,) Shimadzu GC17A – ECD (1999)… conducted many studies of pesticides analysis, multi-residues pesticides analysis using Hewlett Packard GC-MS 5890 (2003), Shimadzu GC – MS QP2010 (2005)
- 2004, NIDQC purchased the first LC-MS, Thermo Finnigan LCQ Advantage MAX LCMS, ion trap, 2009 purchased Thermo – Finnigan TSQ Quantum Ultra Triple Quadrupoles;
- 2010 – 2020: NIDQC & IDQC HCMC purchased more than 20 LC – MS (IT-TOF, Triple Quadrupoles, Q-Trap, Q-Tof)…etc…

Development of HPLC, LC MS in drug quality control and in chemistry research, for the last 30 years before

PGS. Doãn Hữu Khắc

Merck -Hitachi, 655-12A,NIDQC, Hanoi 1987

PGS. TS. Trịnh Văn Quỳ

Hewlett-Packard HP-1100 NIDQC, Hanoi 1996

PGS. TS. Trịnh Văn Lẩu

Thermo Finnigan LCQ Advantage MAX, NIDQC, Hanoi, 2004

PGS. TS. Trịnh Văn Lẩu

Thermo – Finnigan TSQ Quantum Ultra Triple Quadrupoles, 2009

2010 – to date many LC-MS/MS triple quad have been equibled in many Labs of Vietnam

GS.VS. Đặng Vũ Minh
GS.TSKH. Trần Văn Sung

Varian 900-MS Series FTICR MS, Institute Of Chemistry, VAST, 2009

GS.TS. Lưu Văn Bôi

Thermo LTQ XL (Orbitrap), Faculty of Chemistry, University of Natural Sciences, 2009

PGS.TS. Trần Việt Hùng

In IDQC HCMC, we have 12 LC-MS from Agilent, Shimadzu, Waters

The last equipped

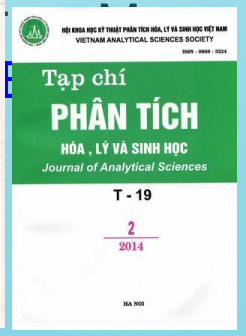
Waters Xevo G2-Xs Qtof, IDQC HCMC 2019

2. Unforgettable memories, grateful to Prof. Pham Hung Viet

- 1995 in Faculty of Chemistry, 17-19 Le Thanh Tong, St; 1996 Prof PH Viet gave lectures of UV-Vis, HPLC and trainings in NIDQC, 48 Hai Ba Trung St., Hanoi
- 2002, participated in the scientific conference held by the Research Center for Environmental Technology and Sustainable Development, on Analysis for Endocrine Disrupying Compouds in the environments – EDCs, Hanoi
- 2003, participated the 10th Asian Chemical Congres, Hanoi
  - Mutiresidues analysis of organochlorine pesticides in Panax ginseng using Solid Phase Micro-Extraction – GC - MS
- After nearly 20 years, honorably invite… 12, 2022, 7th ANALYTICA VIETNAM …

Feb., 2020, VAST, 18 Hoang Quoc Viet St.,

Published papers since 1998…



Journal of Analytical Science, Chemistry, Physic and Biology

Analyst in the field of drug quality control, but most of the experimental works for 20 years 1996 – 2016 are listed below:

Drug quality control, assay, qualitative, quantitative ➔ precise, accurate amount to 10^{-4} g
Limit test in Drug quality control, food safety analysis, environment analysis ➔ trace analysis 10^{-9} to 10^{-4} g

- Anti-counterfeiting and commercial fraud (chemical drugs illegally added in traditional medicines, dietary supplements…: corticoids, antidiabetics, PDE5 inhibitors analogues, sibutramine (2004 – 2010, 2018, 2019);
- Residues of antibiotics (chloramphenicol, streptomycin)...in seafood, aquaculture (2005 - 2007);
- Analysis of aflatoxin, mycotoxins in food and herb, pharmaceutical materials (2002 – 2005);
- Analysis of melamine in milk and dairy products (2005 – 2006);
- Residues of clenbuterol, salbutamol in pork meat (2006 – 2007),

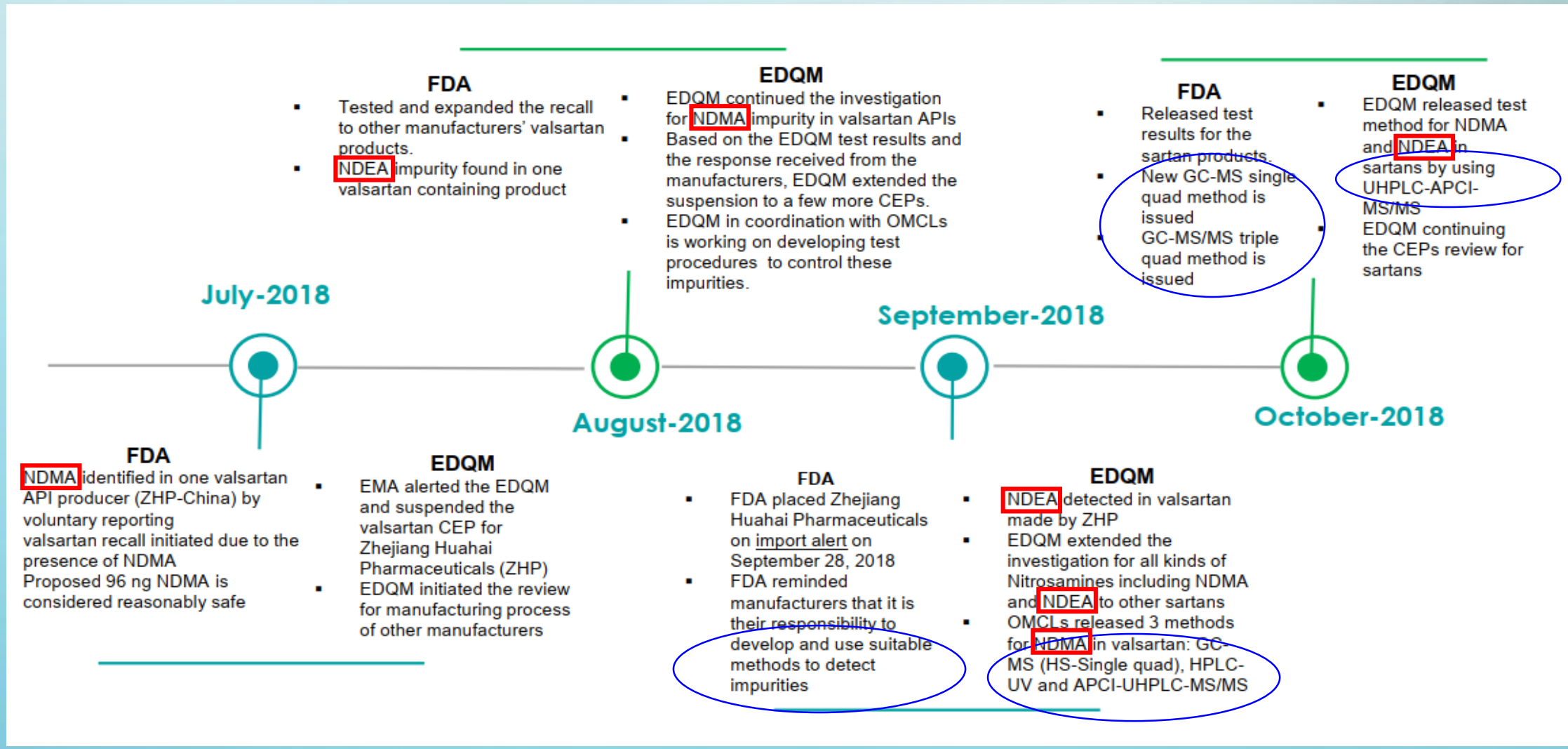
Analyst in the field of drug quality control, but most of the experimental works for 20 years 1996 – 2016 are listed below:

- Herbicide residues 2,4 - D, 2,4,5 – T… (2004-2005)
- Pesticides (organic chlorine, organophosphorus, pyrethroid) in medicinal herbs, herbal products... (1999 – 2005)
- Analysis of drugs in biological fluids during new drug development, for clinical evaluation and monitoring, bio equivalence (BE)…(2006 – to date)
- In forensic toxicology research: analysis of synthetic drugs of amphetamine group (ATS), Narcotic addictive group (2008 – to date);
- Analysis of doping substances in sports (2009 – 2011);
- Analysis of war poison dioxin…etc

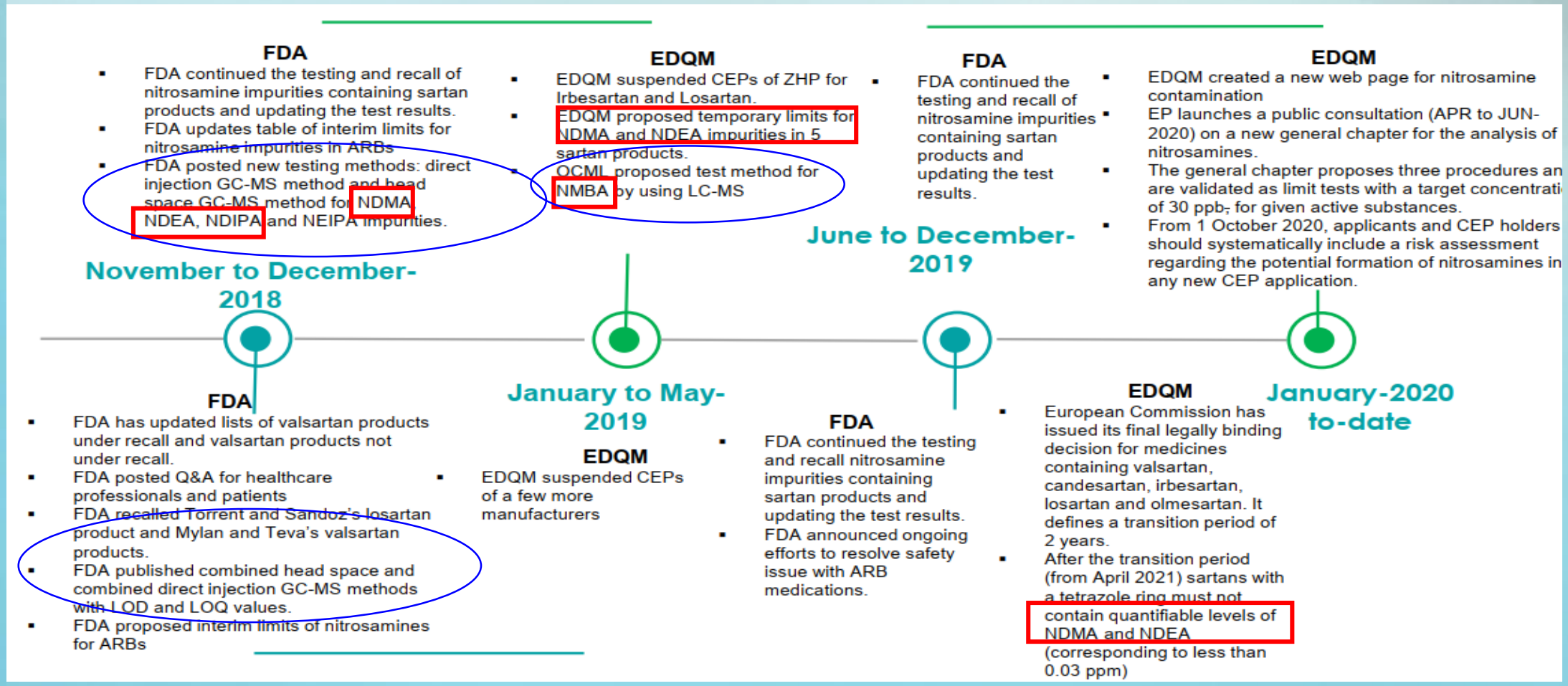
3. Brief report of history and analytical method for nitrosamine impurities control in the Words and in Vietnam

NEW LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR QUALITY CONTROL OF NITROSAMINE IMPURITIES (NDMA, NDEA, AND NMBA) IN SOME PHARMACEUTICAL PRODUCTS (VALSARTAN, IRBESARTAN, LOSARTAN, CANDESARTAN, AND TELMISARTAN TABLETS)

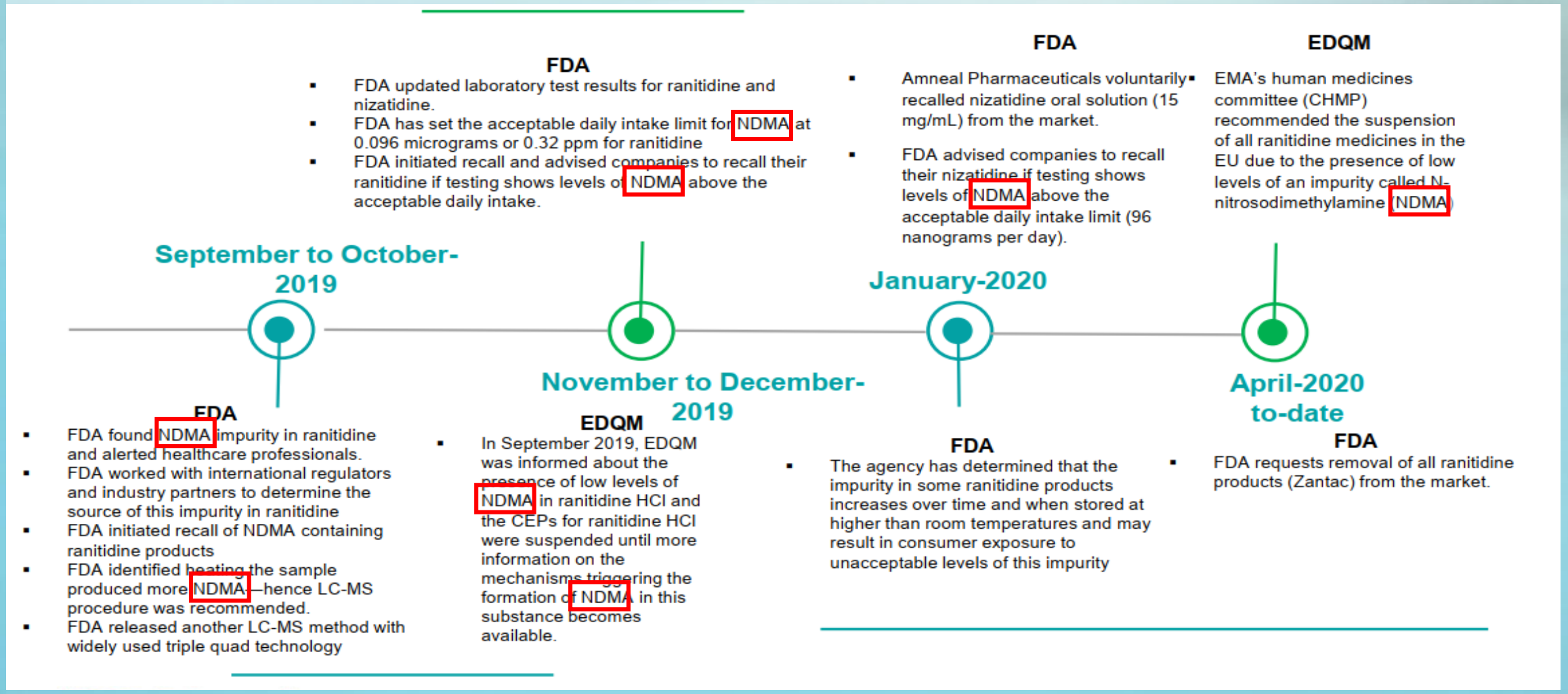
- # History of nitrosamine impurities Regulations of FDA and EDQM



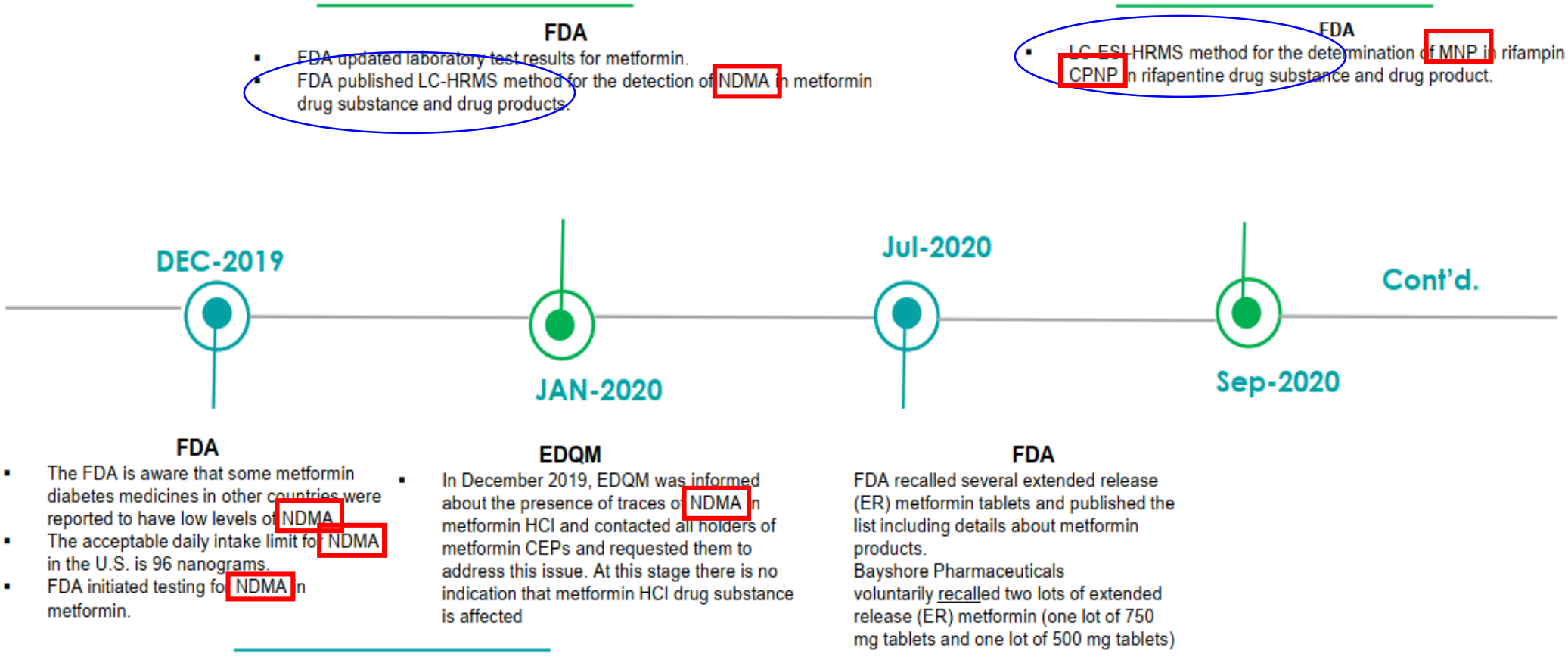
- # History of nitrosamine impurities Regulations of FDA and EDQM



- # FDA and EQDM Regulations on Ranitidine and Nizatidine



• FDA and EQDM Regulations on Metformin



- **Regulations on management of Nitrosamine impurities in Sartan of Drug Administration of Vietnam, DAV- CV, *5853/QLD-CL, controlling API ingredients for manufacturing sartans finished products on April 19, 2019***

Max intake Acceptable intake Limit Acceptable intakec Limit Acceptable intakec Limit

Substances

Tên dược chất	Liều tối đa hàng ngày (mg/ngày)	Lượng NDMA chấp nhận (ng/ngày)	Giới hạn NDMA (ppm)	Lượng NDEA chấp nhận (ng/ngày)	Giới hạn NDEA (ppm)	Lượng NMBA chấp nhận (ng/ngày)	Giới hạn NMBA (ppm)
Valsartan	320	96	0,3	26,5	0,083	96	0,3
Losartan	100	96	0,96	26,5	0,27	96	0,96
Irbesartan	300	96	0,32	26,5	0,088	96	0,32
Azilsartan	80	96	1,2	26,5	0,33	96	1,2
Olmesartan	40	96	2,4	26,5	0,66	96	2,4
Eprosartan	800	96	0,12	26,5	0,033	96	0,12
Candesartan	32	96	3,0	26,5	0,83	96	3,0
Telmisartan	80	96	1,2	26,5	0,33	96	1,2

(Cục Quản lý Dược sẽ tiếp tục cập nhật về giới hạn các tạp chất khác thuộc nhóm nitrosamine sau khi có kết quả đánh giá nguy cơ).

- **Regulations on management of Nitrosamine impurities in Sartan of Drug Administration of Vietnam, documentary DAV- CV, 16813/QLD-CL;**
  Report on detection of ranitidine-containing drugs containing NDMA impurities exceeding the allowable limit on October 2, 2019

In case the batch of Ranitidine pharmaceutical ingredients contains NDMA impurities, the allowable limit of NDMA impurities must not be exceeded according to the acceptance limit specified in ICH M7; specifically: The temporary acceptable NDMA limit should not exceed 0.32ppm (calculated on the maximum acceptable dose of NDMA is 96/nanogram/day and the maximum using dose of Ranitidine is 300mg/day).

- **Regulations on NDMA in Metformin, documentary DAV-CV 297/QLD-CL Metformin quality control dated January 25, 2021**

The only batches of pharmaceutical ingredients Metformin may be put into production, which meet quality standards and contain NDMA impurities that must not exceed the allowable limit of NDMA impurities according to the acceptance limit specified in ICM M7: The temporary acceptable NDMA limit should not exceed 0.32ppm (calculated on the maximum acceptable dose of NDMA is 96 nanogram/day).

Nitrosamine impurity analysis methods

- In the world
  - **US Pharmacopoeia (USP 43)**: Analysis of nitrosamines in the Sartan group (API and finished products): GC MS/MS and HPLC MS/MS
  - **British Pharmacopoeia (BP 2021)**: Analysis of nitrosamines in the group of Sartans (API and finished products): GC MS/MS and HPLC MS/MS; methods-Singapore: Analysis of NDMA in metformin (API) and finished products: GC MS/MS

USP 2021

4 proceduce: effective December 1, 2021

-Proceduce 1: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, NMPA, and NDBA in selected sartans (valsartan, irbesartan, and losartan potassium) by HPLC–HRMS

-Proceduce 2: Quantitation of NDMA, NDEA, NDIPA, and NEIPA in selected sartans (valsartan, irbesartan, losartan potassium, olmesartan medoxomil, candesartan cilexetil, and telmisartan) by headspace GC–MS

-Proceduce 3: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMBA, and NDBA in selected sartans (valsartan, losartan potassium, olmesartan medoxomil, candesartan cilexetil, and telmisartan) by HPLC–MS/MS

-Proceduce 4: Quantitation of NDMA, NDEA, NDIPA, NEIPA, NMPA, and NDBA in selected sartans (valsartan, losartan potassium, and candesartan cilexetil) by GC–MS/MS (triple-quad)

BP 2021

3 proceduce:

- Proceduce A (LC MS/MS)
- Proceduce B (GC MS)

limit test (30ppb)

- Proceduce C : quantitative test

Table 2.5.42.-1. – *Scope of the validation*

Active substance (monograph number)	NDMA	NDEA	NDBA	NMBA	NDiPA	NEiPA	NDPA
Candesartan cilexetil (2573)	A*BC	ABC	C	A	AC	AC	C
Irbesartan (2465)	A*BC	ABC	C	A	AC	AC	C
Losartan potassium (2232)	A*BC	ABC	C	A	AC	AC	C
Olmesartan medoxomil (2600)	A*BC	ABC	C	A	AC	AC	C
Valsartan (2423)	A*BC	ABC	C	A	AC	AC	C

Nitrosamine impurity analysis methods in Vietnam

- NIDQC, Hanoi, MOH (ISO/IEC 17025, GLP, WHO Prequalified Labs)
  - NDMA, NDEA: GS-MS/MS, headspace and direct injection),
  - NMBA: LC-MS/MS

- IDQC HCMC, HoChiMinh City, MOH (ISO/IEC 17025, GLP, WHO Prequalified Labs)

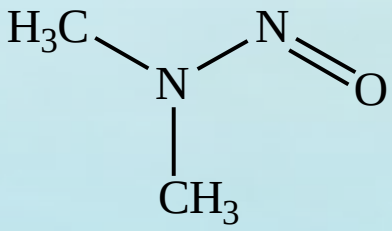
  NDMA, NDEA, NMBA: LC-MS/MS simultaneous quantitation

- **TSL** Testing Center (ISO/IEC 17025)

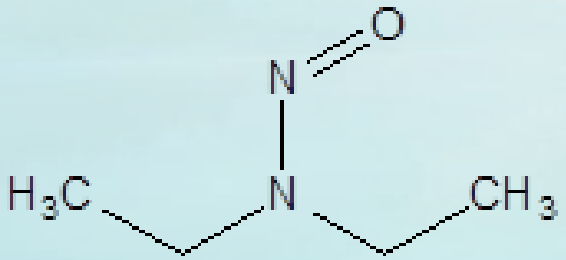
  Not reveal, may be using USP 43 Monographs, GC-MS/MS and LC-MS/MS

IDQC HCM Method for nitrosamine impurities (NDMA, NDEA and NMBA) in some pharmaceutical products

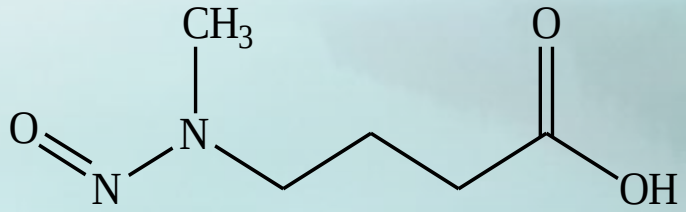
[Genotoxic Impurity]



N-nitrosodimethylamine, **NDMA**
$C_2H_6N_2O$, 74.08 g/mol



N-nitrosodiethylamine, **NDEA**
$C_4H_{10}N_2O$, 102.13 g/mol



N-Nitroso-N-Methyl-4-Amino Butyric Acid, **NMBE**
146.14g/mol

Chromatographic conditions

- UPLC Waters (USA) chromatography system composed by: binary solvent manager ACQUITY I, mass spectrometry detector (Xevo TQ-S Micro); sample manager FTN-I and software controller MassLynx 4.1

Chromatographic conditions

Mobile phase: a gradient programme is as follows:

Time (min)	10 mM acetate buffer solution pH 3.0 (%)	Acetonitrile (%)
0	98	2
5	98	2
9	40	60
10	98	2
13	98	2

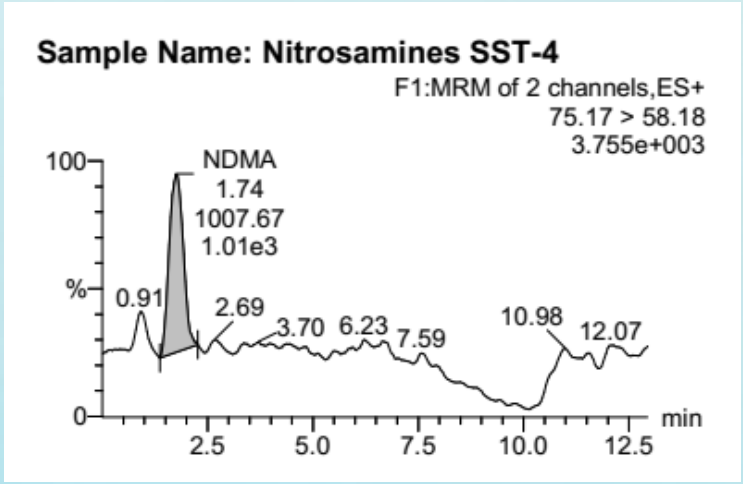
MS conditions

Ionization: Electrospray Ionization (ESI), Scan settings: See Table

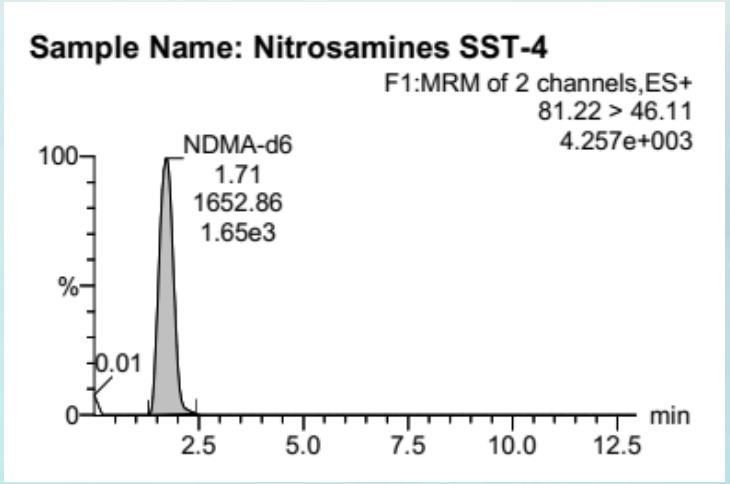
Nitrosamine impurity	Acquisition mode	Polarity	Transitions	Collision energy (V)	Cone voltage (V)
			MRM-1		
NDMA	MRM	Positive	75.17 amu → 58.18 amu	8	28
NDEA	MRM	Positive	103.09 amu → 28.90 amu	10	28
NMBA	MRM	Positive	147.19 amu → 44.14 amu	11	8
NDMA-d6	MRM	Positive	81.22 amu → 46.11 amu	12	28

Table 1. Limit of sartan impurities, sample weight, dilution factor and test concentration of relevant drug substance

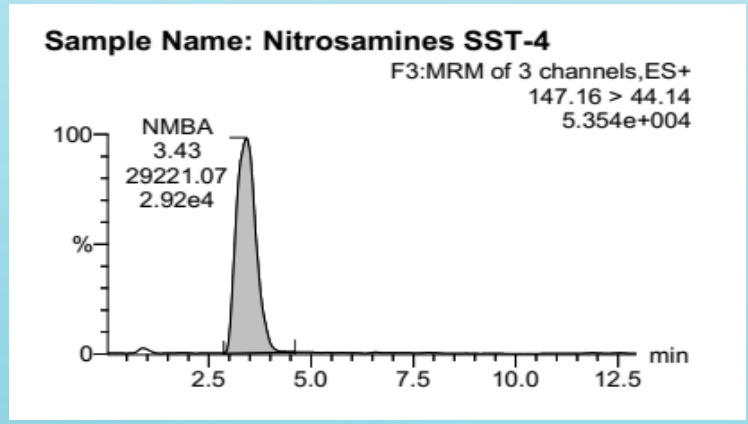
Drug substance	Impurity	Limit (ppm) (*)	Sample weight m (mg) /Dilution factor V (ml)	Test concentration (ng/ml)
Valsartan	NDMA	0.3	1000 mg/ 7.5 ml	40
	NDEA	0.083		11.1
	NMBA	0.3		40
Losartan	NDMA	0.96	625 mg/ 15 ml	40
	NDEA	0.27		11.2
	NMBA	0.96		40
Irbesartan	NDMA	0.32	625 mg/ 5 ml	40
	NDEA	0.088		11.0
	NMBA	0.32		40
Candesartan	NDMA	3.0	200 mg/ 15 ml	40
	NDEA	0.83		11.1
	NMBA	3.0		40
Telmisartan	NDMA	1.2	500 mg/ 15 ml	40
	NDEA	0.33		11.0
	NMBA	1.2		40



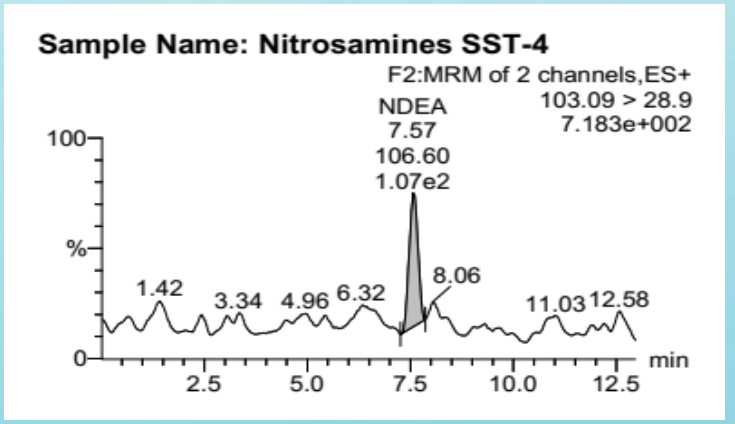
A



B



C



D

Fig. 2. Chromatograms obtained for the system suitability test. Injections of: **A**, NDMA; **B**, NDMA-d6; **C**, NMBA; **D**, NDEA.

Table 2, 3. System suitability testing results, retention time ratio of standard and sample

No.	Peak response ratio of NDMA/NDEA/NMBA to NDMA-d6			S/N			
	NDMA	NDEA	NMBA	NDMA	NDEA	NMBA	NDMA-d6
1	0.632	0.065	17.571	12.94	6.32	359.42	4470.00
2	0.656	0.063	18.771	18.90	7.39	828.55	4712.00
3	0.633	0.061	17.619	10.67	15.55	519.74	4376.00
4	0.610	0.064	17.679	18.77	7.39	835.17	4723.00
5	0.653	0.063	18.630	12.86	6.14	358.13	4472.00
6	0.601	0.068	17.931	10.92	16.31	514.46	4367.00
Average	0.631	0.064	18.034	14.18	9.85	569.25	4520.00
RSD (%)	3.53	3.54	2.96				

No.	Retention time ratio of NDMA/NDEA/NMBA to NDMA-d6 in sample chromatograms of valsartan			Retention time ratio of NDMA/NDEA/NMBA to NDMA-d6 in standard chromatograms of valsartan		
	NDMA	NDEA	NMBA	NDMA	NDEA	NMBA
1	1.045	4.459	2.012	1.066	4.459	2.012
2	1.022	4.459	2.035	1.045	4.459	2.012
3	1.022	4.459	2.035	1.045	4.459	2.012
4	1.022	4.459	2.012	1.022	4.437	2.012
5	1.022	4.459	2.035	1.045	4.459	2.012
6	1.022	4.459	2.035	1.045	4.459	2.012
Average	1.026	4.459	2.027	1.044	4.456	2.012
RSD (%)	0.81	0.00	0.57	1.33	0.20	0.00
Deviation (%)	1.75	0.07	0.74			

Table 4. Limit of detection (ppm) of NDMA, NDEA, and NMBA

Drug substance	NDMA		NDEA		NMBA	
	Drug product	Drug substance	Drug product	Drug substance	Drug product	Drug substance
Valsartan	0.0080	0.0080	0.0022	0.0022	0.0080	0.0080
Irbesartan	0.0080	0.0080	0.0022	0.0022	0.0080	0.0080
Losartan	0.0080	0.0080	0.0022	0.0022	0.0080	0.0080
Candesartan	0.0080	0.0080	0.0022	0.0022	0.0080	0.0080
Telmisartan	0.0080	0.0080	0.0022	0.0022	0.0080	0.0080

Table 6. Results of repeatability precision

	Valsartan drug product			Valsartan drug substance		
	Peak response ratio of of NDMA/NDEA/NMBA to NDMA-d6					
No. of sample solution	NDMA	NDEA	NMBA	NDMA	NDEA	NMBA
1	0.280	0.040	10.789	0.246	0.018	8.194
2	0.308	0.035	10.222	0.200	0.019	7.802
3	0.276	0.037	10.214	0.188	0.016	8.050
4	0.334	0.044	11.268	0.254	0.018	8.298
5	0.306	0.037	10.146	0.193	0.015	7.946
6	0.273	0.039	10.034	0.200	0.020	7.643
Average (1-6)	0.296	0.039	10.445	0.213	0.017	7.989
RSD (%) (1-6)	8.08	7.77	4.60	13.47	9.48	3.05
	Irbesartan drug product			Ibersartan drug substance		
	Peak response ratio of of NDMA/NDEA/NMBA to NDMA-d6					
No. of sample solution	NDMA	NDEA	NMBA	NDMA	NDEA	NMBA
1	0.150	0.015	5.007	0.220	0.019	7.307
2	0.168	0.016	4.823	0.243	0.017	7.679
3	0.174	0.015	5.015	0.254	0.016	7.543
4	0.169	0.017	4.777	0.243	0.017	7.719
5	0.141	0.015	4.937	0.227	0.019	7.978
6	0.142	0.016	5.173	0.222	0.020	7.398
Average (1-6)	0.157	0.016	4.955	0.235	0.018	7.604
RSD (%) (1-6)	9.44	5.16	2.90	5.85	8.38	3.18

Table 6. Results of repeatability precision

	Candesartan drug product			Candesartan drug substance		
	Peak response ratio of of NDMA/NDEA/NMBA to NDMA-d6					
No. of sample solution	NDMA	NDEA	NMBA	NDMA	NDEA	NMBA
1	0.205	0.023	7.311	0.169	0.023	6.961
2	0.222	0.024	7.358	0.161	0.019	6.904
3	0.205	0.027	8.200	0.164	0.023	6.848
4	0.201	0.027	8.373	0.181	0.018	6.459
5	0.205	0.028	8.057	0.229	0.019	6.749
6	0.223	0.024	7.329	0.181	0.017	6.413
Average (1-6)	0.210	0.026	7.771	0.181	0.020	6.723
RSD (%) (1-6)	4.72	7.03	6.32	13.70	13.14	3.47
	Telmisartan drug product			Telmisartan drug substance		
	Peak response ratio of of NDMA/NDEA/NMBA to NDMA-d6					
No. of sample solution	NDMA	NDEA	NMBA	NDMA	NDEA	NMBA
1	0.161	0.017	5.000	0.244	0.022	8.230
2	0.163	0.015	5.229	0.243	0.023	8.048
3	0.166	0.014	4.874	0.248	0.025	7.991
4	0.158	0.019	5.089	0.219	0.023	7.675
5	0.163	0.014	4.903	0.253	0.025	8.008
6	0.149	0.014	4.696	0.242	0.028	8.706
Average (1-6)	0.160	0.015	4.965	0.241	0.025	8.110
RSD (%) (1-6)	3.77	12.69	3.73	4.88	8.64	4.23
	Losartan drug product			Losartan drug substance		
	Peak response ratio of of NDMA/NDEA/NMBA to NDMA-d6					
No. of sample solution	NDMA	NDEA	NMBA	NDMA	NDEA	NMBA
1	0.326	0.029	11.350	0.190	0.019	7.270
2	0.370	0.035	12.000	0.178	0.022	6.832
3	0.359	0.031	11.612	0.191	0.019	7.218
4	0.295	0.035	11.510	0.148	0.020	6.655
5	0.290	0.030	11.243	0.179	0.022	6.774
6	0.318	0.038	11.557	0.157	0.019	6.706
Average (1-6)	0.326	0.033	11.545	0.174	0.020	6.909
RSD (%) (1-6)	10.09	10.80	2.26	10.12	6.80	3.86

Table 7. LOD of NDMA, NDEA, and NMBA in sartan drug substance as well as tablets using various analytical procedures (**LOD obtained from the proposed method*)

NMBA

No.	Method	Analytical Instrument	Drug Product, LOD (ppm)	Drug Substance, LOD (ppm)
1	LC-MS (ESI, Positive)	Acquity UPLC I-Class Plus + Xevo TQ-S Micro	0.0080*	0.0080*
2	LC-MS/MS (APCI, Positive)	Shimadzu HPLC + Sciex QTrap 5500	NA	0.0086 (10)
3	LC-HRMS	HPLC or UHPLC system equipped with temperature-controlled autosampler and column compartment Q ExactiveTM hybrid quadrupole-orbitrap mass spectrometer or Q ExactiveTM HF-X hybrid quadrupole-orbitrap mass spectrometer (ThermoFisher Scientific)	0.01 (8)	0.01 (8)
4	RapidFire-MS/MS	Mass Spectrometry (Agilent 6460C)	0.05 (9)	0.05 (9)

NDMA

No.	Method	Analytical Instrument	Drug Product, LOD (ppm)	Drug Substance, LOD (ppm)
1	LC-MS (ESI, Positive)	Acquity UPLC I-Class Plus + Xevo TQ-S Micro	0.0080*	0.0080*
2	HS-GC-MS	Shimadzu GC-2010Plus + GC-MS QP2020 + HS-Auto-sampler	0.02 (3)	0.02 (3)
3	LC-MS/MS	Agilent Infinity 1290 UHPLC + 6460 APCI-QQQ-MS	0.05 (11)	0.10 (11)
4	GC/MS-HS	Agilent 7890B GC + Agilent 5977A MSD + Agilent 7697A HS Auto-sampler	NA	0.05 (4)
5	GC/MS-HS	Agilent 7890B GC + Agilent 5977A MSD + Agilent 7697A HS Auto-sampler	NA	0.005 (2)
6	GC-MS/MS	Gas Chromatograph with Liquid Autosampler and a Triple Quadrupole Mass Selective Detector	0.015 (5)	0.010 (5)
7	GC-MS/MS	Gas Chromatograph with Liquid Autosampler and a Triple Quadrupole Mass Selective Detector	0.008 (6)	0.005 (6)
8	GC-MS/MS	Gas Chromatography System with a Quadrupole Mass Spectrometry Detector and Headspace Auto-sampler	0.01 (7)	0.01 (7)
9	LC-HRMS	HPLC or UHPLC system equipped with temperature-controlled autosampler and column compartment Q ExactiveTM hybrid quadrupole-orbitrap mass spectrometer or Q ExactiveTM HF-X hybrid quadrupole-orbitrap mass	0.005 (8)	0.005 (8)

Table 7. LOD of NDMA, NDEA, and NMBA in sartan drug substance as well as tablets using various analytical procedures (**LOD obtained from the proposed method*)

NDEA				
No.	Method	Analytical Instrument	Drug Product, LOD (ppm)	Drug Substance, LOD (ppm)
1	LC-MS (ESI, Positive)	Acquity UPLC I-Class Plus + Xevo TQ-S Micro	0.0022*	0.0022*
2	GC-MS/MS	Agilent 7890B_7000D	0.005 (1)	0.005 (1)
3	LC-MS/MS	Agilent Infinity 1290 UHPLC + 6460 APCI-QQQ-MS	0.02 (11)	0.04 (11)
4	GC/MS-HS	Agilent 7890B GC + Agilent 5977A MSD + Agilent 7697A HS Auto-sampler	NA	0.02 (2)
5	HS-GC-MS	Shimadzu GC-2010Plus + GC-MS QP2020 + HS-Auto-sampler	0.02 (3)	0.02 (3)
6	GC-MS/MS	Gas Chromatograph with Liquid Autosampler and a Triple Quadrupole Mass Selective Detector	0.015 (5)	0.010 (5)
7	GC-MS/MS	Gas Chromatograph with Liquid Autosampler and a Triple Quadrupole Mass Selective Detector	0.002 (6)	0.001 (6)
8	GC-MS/MS	Gas Chromatography System with a Quadrupole Mass Spectrometry Detector and Headspace Auto-sampler	0.01 (7)	0.01 (7)
9	LC-HRMS	HPLC or UHPLC system equipped with temperature-controlled autosampler and column compartment Q ExactiveTM hybrid quadrupole-orbitrap mass spectrometer or Q ExactiveTM HF-X hybrid quadrupole-orbitrap mass spectrometer (ThermoFisher Scientific)	0.016 (8)	0.016 (8)

CONCLUSION

- Under the conditions described, the New Liquid Chromatography Tandem Mass Spectrometry Method has been developed to simultaneously quantify NDMA, NDEA, and NMBA in the “sartan” tablets for Quality Control purpose. The method was fully validated according to the International Conference on Harmonisation guideline ICH (Q2R1) on Validation of Analytical Procedures and was determined to be repeatable and specific as a analytical procedure for impurity limit test. The detection limit for NDEA and NMBA met the requirement of the Drug Administration of Vietnam. The method of analysis can be useful reference material by laboratories and regulatory agencies including pharmacopoeia commissions in quality control or developing monographs.

	NDQC	IDQC HCM
Method	Analysis NDMA, NDEA using GC-MS headspace or GC-MS/MS direct injection, and analysis NMBA by LC-MS-MS	Simultaneous analysis of NDMA,NDEA, NMBA bằng LC-MS/MS
Instruments	NDMA, NDEA GC-MS: Agilent 7890 GC with Agilent 5977 A MSD and Agilent 7697 A HS Autosampler NMBA LC-MS: UPLC Waters with Xevo TQD and ESI Soucre	UPLC AQUITY Class I WATERS Detector TQS Micro ESI Source
Sample preparation	GC MS Headspace: 500 mg API + 5ml NMP into 20 ml vial headspace, vortex; GC MSMS Direct Injection: 500 mg API + 5 ml IS (NDMA C13-d6) + 2 ml MeCl2, vortex 1 min, centrifuge 4000 rpm for 2.5 min; LCMS (NMBA) : 500 mg API + 2ml IS NDMd6 Stock+ 5 ml Diluent (MeOH- H2O =1:1), SA 30 min, centrifuge 900 rpm x 10 min, filter through 0,2 um membrane	Weigh x mg API + V ml Diluent (buffer –ACN 98:2), => NDMA,NDEA, NMBA concentrations are 40,11,40 ng/ml, sonicate for15 min, centrifuge 4000 rpm x 5 min. pipet 2 ml + 150 ul IS NDMA-d6 lọc qua màng lọc 0,22 um
Chromatographic condition	**NDMA NDEA** GC MS Headspace **NDMA NDEA** GC MS MS injection **NMBA** LC MS/MS	NDMA NDEA NMBA UPLC MS/MS: Column: HSS T3 C18 (100 x 2.1; 1.7 um); Mobile phase A: amoni acetate 10 mM pH 3.0; Mobile phase B: Acetonitril; Flow rate: 0,3 ml/min; Injection volume: 50 ul Autosampler: 15 oC

GC/MS - HS Parameters	
Instrument:	Agilent 7890B GC with Agilent 5977A MSD and Agilent 7697A HS Auto-sampler
Column:	DB-1701, 30 m x 0.25 mm, 1.00 µm (PN: 122-0733), or equivalent
Inlet Temperature:	220 °C
Column Flow:	1 mL/min
Split Ratio	5:1
Oven Program:	40 °C for 0.5 min.; 20 °C/min to 160 °C, hold for 0 min; 10 °C/min to 240 °C, hold for 2 min.
GC Run Time	16.5 min.
GC Cycle Time:	25 min.

Gas Chromatograph (GC) Conditions	
Inlet Temperature	250 °C
Transfer line Temperature	250 °C
Injection Type	Pulsed Splitless: 12.285 psi until 0.5min
Injection Volume	2 µL
Flowrate	1 mL/min
Oven Program	40 °C for 0.5 min→200 °C at 20 °C/min→250 °C at 60 °C/min and hold for 3 min
Runtime	12.33 min

[1.7. Mass spectrometer conditions

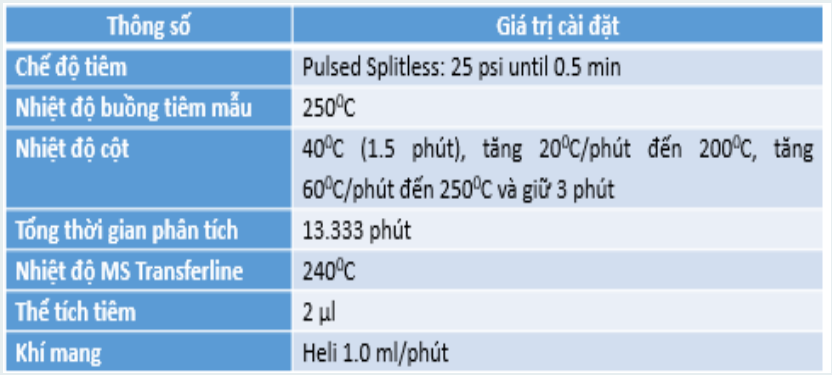
- *Instrument:*
  Waters Xevo TQD system with ESI source.
- *Ion Source Settings:*
  - Ion Source: ESI
  - Capillary (kV): 3.0
  - Desolvation temp.: 400°C
  - Gas flow (l/hr): 700
- *Scan Settings*
  - Polarity: positive ion;
  - Scan type: MRM;

	Precursor ion	Cone (V)	Product Ion	Collision Energy (V)
NMBA	146.9	14	117.0	6
NDMA-d6	80.9	23	46.0	23

- NDMA: 75,17 → 58,18; Cone voltage: 28 V; Collision energy: 8V
- NDEA: 103,09 → 28,90; Cone voltage: 26 V; Collision energy: 10V
- NMBA: 147,16 → 44,14; Cone voltage: 8 V; Collision energy: 11V
- NDMA-d6: 81,22 → 46,11; Cone voltage: 28 V, Collision energy: 12V
- ESI Positive
- Điện áp mao quản: 3,5 kV
- Desolvation Gas Flow: 950 L/Hr
- Cone Gas Flow: 150 L/Hr

Thời gian (phút)	Dung môi A	Dung môi B
0	98	2
5	98	2
9	40	60
10	98	2
13	98	2

Analysis of nitosamine impurities by NIDQC and IDQC HCMC, Metformin

	NIDQC	IDQC HCM
Method	LC-MS/MS	LC-MS/MS
Instruments	GC-MS: Agilent 7890 GC with Agilent 5977 A MSD and Agilent 7697 A HS Autosampler	Hệ thống UPLC AQUITY Class I WATERS Đầu dò Xevo TQS Micro ESI Source
Sample preparation	*2.2.2.2.Phương pháp chuẩn bị mẫu:* - *Dung môi*: Diclorometan - *Chuẩn nội*: NDMA – d6 nồng độ 50 ng/ml trong HCl 1N. - *Mẫu chuẩn*: Từ chuẩn gốc NDMA 5000 ppm trong methanol và NDEA 5000 ppm trong methanol pha loãng với diclorometan để được dãy chuẩn hỗn hợp có nồng độ NDMA và NDEA là: 100, 80, 40, 20, 10, 5 và 2,5 ng/ml có sẵn NDMA- d6 nồng độ 50 ng/ml làm chuẩn nội. - *Mẫu thử (nguyên liệu)*: Cân chính xác khoảng 500 mg Metformin vào lọ thủy tinh nắp xoáy dung tích 33 ml. Thêm chính xác 5,0 ml chuẩn nội (NDMA –d6 nồng độ 50 ng/ml trong HCl 1N). Lắc xoáy 1 phút. Sau đó thêm 5,0 ml diclorometan, lắc xoáy 2 phút. Ly tâm 3500 vòng/ phút trong 5 phút. Kết quả thu được là lớp nước bên trên và lớp dung môi hữu cơ ở phía dưới. Dùng pipette hút khoảng 2 ml diclorometan ở lớp dưới, lọc qua màng lọc 0,45 µm và chuyển vào vial đem phân tích. - *Mẫu thử (chế phẩm)*: Lấy 10 viên nghiền thành bột mịn. Cân 1 lượng mẫu thử tương ứng với 500 mg Metformin vào lọ thủy tinh nắp xoáy dung tích 33 ml. Thêm chính xác 5,0 ml chuẩn nội (NDMA –d6 nồng độ 50 ng/ml trong HCl 1N). Lắc xoáy 1 phút. Sau đó thêm 5,0 ml diclorometan, lắc xoáy 2 phút. Ly tâm 3500 vòng / phút trong 5 phút. Kết quả thu được là lớp nước bên trên và lớp dung môi hữu cơ ở phía dưới. Dùng pipette hút khoảng 2 ml diclorometan ở lớp dưới, lọc qua màng lọc 0,45 µm và chuyển vào vial đem phân tích. Song song chuẩn bị mẫu spike tương tự, dùng dung dịch chuẩn NDMA 3,2 ng/ml, NDEA nồng độ 8,8 ng/ml, chuẩn nội NDMA – d6 nồng độ 50 ng/ml chuẩn bị trong HCl 1N để đánh giá hiệu suất phương pháp	- Đối với mẫu nguyên liệu: Cân chính xác khoảng 3,0000 g nguyên liệu metformin vào bình nón nút mài 50 ml, thêm chính xác 10 ml nước, lắc siêu âm 15 phút, để nguội, ly tâm dung dịch thu được với tốc độ 4000 vòng/phút trong 5 phút. Hút chính xác 2 ml dung dịch ly tâm trên vào ống nghiệm thủy tinh, thêm chính xác 100 µl nội chuẩn IS1 vào ống nghiệm trên, lắc xoáy 1 phút. Nạp 1 ml dung dịch trên lên cột SPE Strata C18-E (55 µm, 70 A); 500mg/6ml, rửa giải với tốc độ 1 giọt/ giây, thu dịch lọc. Tiếp tục rửa giải lặp lại như trên với 1 ml dung môi A, gộp các dịch lọc và lọc qua màng lọc 0,22 µm. - Đối với mẫu thuốc viên nén: Cân khối lượng của 20 viên, nghiền mịn, trộn đều. Cân khối lượng bột thuốc tương ứng 3,0000 g metformin cho vào bình nón nút mài 50 ml, thêm chính xác 10 ml nước , siêu âm 15 phút. Ly tâm dung dịch thu được với tốc độ 4000 vòng/phút trong 5 phút. Hút chính xác 2 ml dung dịch ly tâm ở trên vào ống nghiệm thủy tinh, thêm chính xác 100 µl nội chuẩn IS1 vào ống nghiệm trên, lắc xoáy 1 phút. Nạp 1 ml dung dịch trên lên cột SPE Strata C18-E (55 µm, 70 A); 500mg/6ml, rửa giải với tốc độ 1 giọt/ giây, thu dịch lọc. Tiếp tục rửa giải lặp lại như trên với 1 ml dung môi A, gộp các dịch lọc và lọc qua màng lọc 0,22 µm.
Chromatographic condition		Column: HSS T3 C18 (100 x 2,1 mm; 1,8 µm); autosampler tem.: 15 ºC Inj.vol: 50 µl; : Gradient

Thông số	Giá trị cài đặt
Chế độ tiêm	Pulsed Splitless: 25 psi until 0.5 min
Nhiệt độ buồng tiêm mẫu	250^{0}C
Nhiệt độ cột	40^{0}C (1.5 phút), tăng 20^{0}C/phút đến 200^{0}C, tăng 60^{0}C/phút đến 250^{0}C và giữ 3 phút
Tổng thời gian phân tích	13.333 phút
Nhiệt độ MS Transferline	240^{0}C
Thể tích tiêm	2 µl
Khí mang	Heli 1.0 ml/phút

Thời gian (phút)	Dung môi A (%)	Dung môi B (%)
0	98	2
4	98	2
7	0	100
15	0	100
16	98	2
21	98	2

Analysis of nitosamine impurities by NIDQC and IDQC HCMC, Ranitidin

	NIDQC	IDQC HCM
Method	LC MSMS	LC MSMS
Instruments	**Sciex Qtrap 6500+**	**UPLC AQUITY Class I WATERS, Xevo TQS Micro ESI Source**
Sample preparation	• **Phân tích tạp NDMA – trong Ranitidin:(tiếp)** + Dung dịch chuẩn nội làm việc: * Chuẩn bị dung dịch chuẩn nội làm việc trong nước có nồng độ NDMA-d6 là 1 µg/mL + Dung dịch chuẩn: * Chuẩn bị dãy các dung dịch chuẩn trong nước có nồng độ NDMA lần lượt là 2, 4, 10, 20, 40 ng/mL và chuẩn nội NDMA-d6 là 5 ng/mL + Dung dịch thử: Cân chính xác khoảng 134 mg nguyên liệu Ranitidin HCl (tương đương 120 mg Ranitidin) vào bình định mức 10 mL. Thêm 6 mL nước, thêm 50µL dung dịch NDMA-d6 nồng độ 1µg/mL. Lắc xoáy cho tan hết. Làm đầy bằng nước	- Đối với mẫu nguyên liệu: Cân chính xác khoảng 1,000 g nguyên liệu ranitidine hoặc metformin vào bình nón nút mài 50 ml, thêm chính xác 10 ml nước, lắc siêu âm 15 phút, để nguội, ly tâm dung dịch thu được với tốc độ 4000 vòng/phút trong 5 phút. Hút chính xác 2 ml dung dịch ly tâm trên vào ống nghiệm thủy tinh, thêm chính xác 100 µl nội chuẩn IS1 vào ống nghiệm trên, lắc xoáy 1 phút. Nạp 1 ml dung dịch trên lên cột SPE Strata C18-E (55 µm, 70 A); 500mg/6ml, rửa giải với tốc độ 1 giọt/ giây, thu dịch lọc. Tiếp tục rửa giải lặp lại như trên với 1 ml dung môi A, gộp các dịch lọc và lọc qua màng lọc 0,22 µm.
Chromatographic condition		- Hệ thống UPLC – MS/MS • Cột: C18 (100 x 3 mm; 3 µm) • Nhiệt độ cột: nhiệt độ phòng • Nhiệt độ autosampler: 15 °C • Thể tích tiêm: 10 µl • Chương trình chạy: Gradient

Cột sắc ký	Agilent Poroshell-C18; 2,7µm; 100*4,6 mm		
Nhiệt độ cột	30°C		
Tốc độ dòng	0,8 mL/phút		
Pha động A	Acid formic 0,1%/Methanol		
Pha động B	Acid formic 0,1%/Nước		
Chương trình gradient	Thời gian (phút)	Pha động A %	Pha động B %
	0	5	95
	1,2	5	95
	3	20	80
	6	100	0
	7,4	100	0
	7,5	5	95
	12	5	95
Thể tích tiêm	10 µL		
Nhiệt độ Autosampler	6 °C		

Thời gian (phút)	Dung môi A (%)	Dung môi B (%)
0	98	2
4	98	2
7	0	100
15	0	100
16	98	2
21	98	2

VIỆN KIỂM NGHIỆM THUỐC THÀNH PHỐ HỒ CHÍ MINH
Institute of Drug Quality Control – HoChiMinh City, Ministry of Health

- **IDQC HCMC Labs equipments, the future of multi-functional QC labs, quality control for food, pharmaceuticals, cosmetics, vaccine and bio-medical products, seeking for research co-operation, support and service from State bodies and private companies**

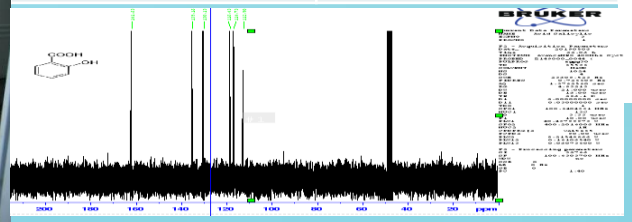
200 Co Bac St, Co Giang Ward, Dít. 1, HoChiMinh City
https://www.vienkiemnghiem.gov.vn; www.niqc.gov.vn

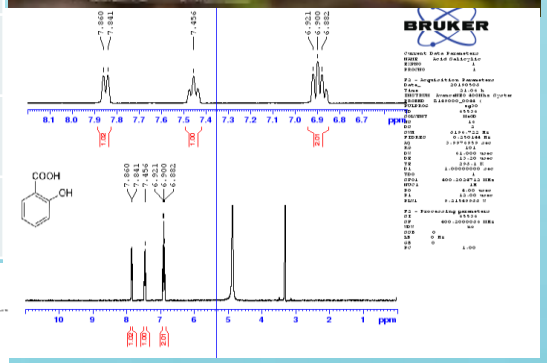
R&D, academic researches: drug discovery & drug development, industrial R&D labo, QC Labs...

Analysis of active ingredient structure (NMR, MS, IR,...), quantification, purity determination

Bruker NMR
Spectrometer 400MHz

Spectra	Times (min)
1H	5
13C	70
DEPT	45
COSY	45
HSQC	45
HMBC	60
NOESY	45
ROESY	60





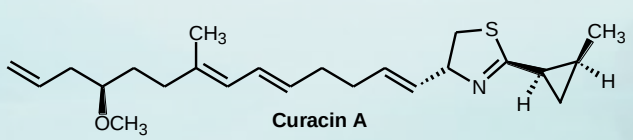
1mg Acid salicyclic/ $MeOD_4$; 400MHz; Prodigy

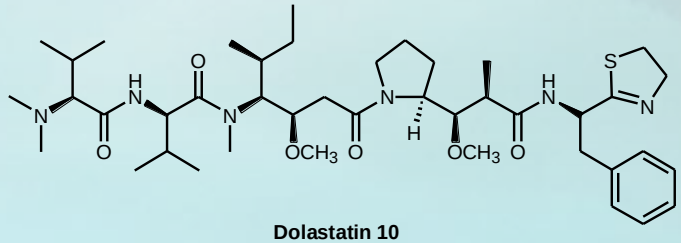
In IDQC HCMC, we have 12 LC-MS from Agilent, Shimadzu, Waters, 65 HPLC with variety of detector

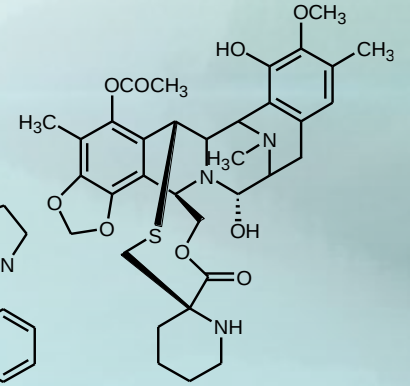
Waters UPLC-QTOF-MS, Xevo G2-Xs Qtof, 2019

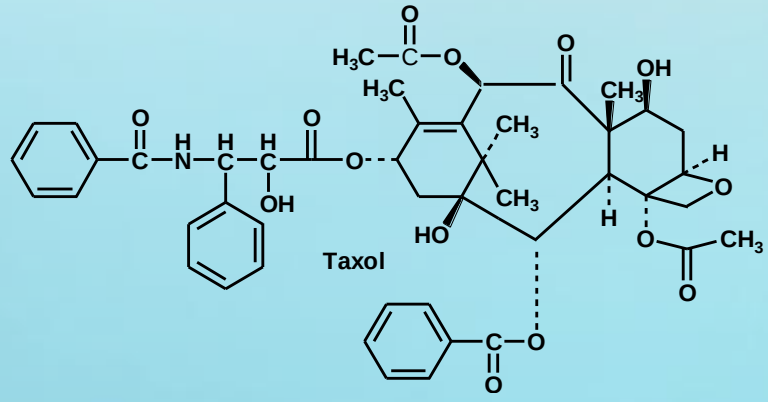
Drug Discovery and Development - DDD

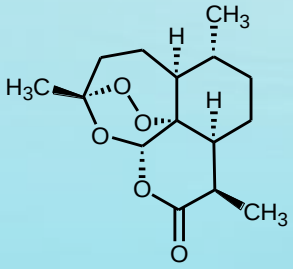
- Drug discovery
  - Chemical synthesis
  - Natural screening
- Drug development





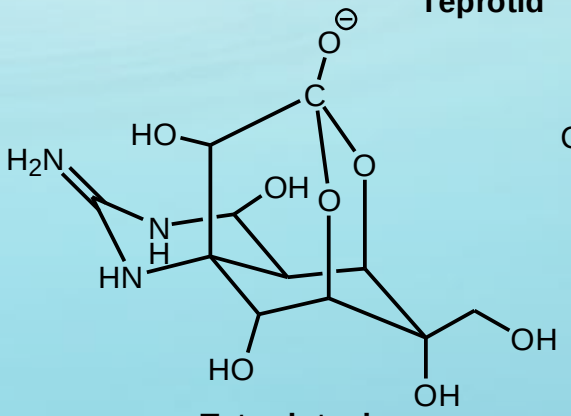


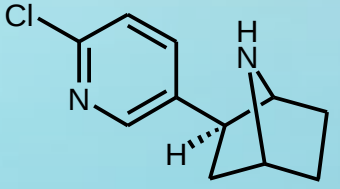




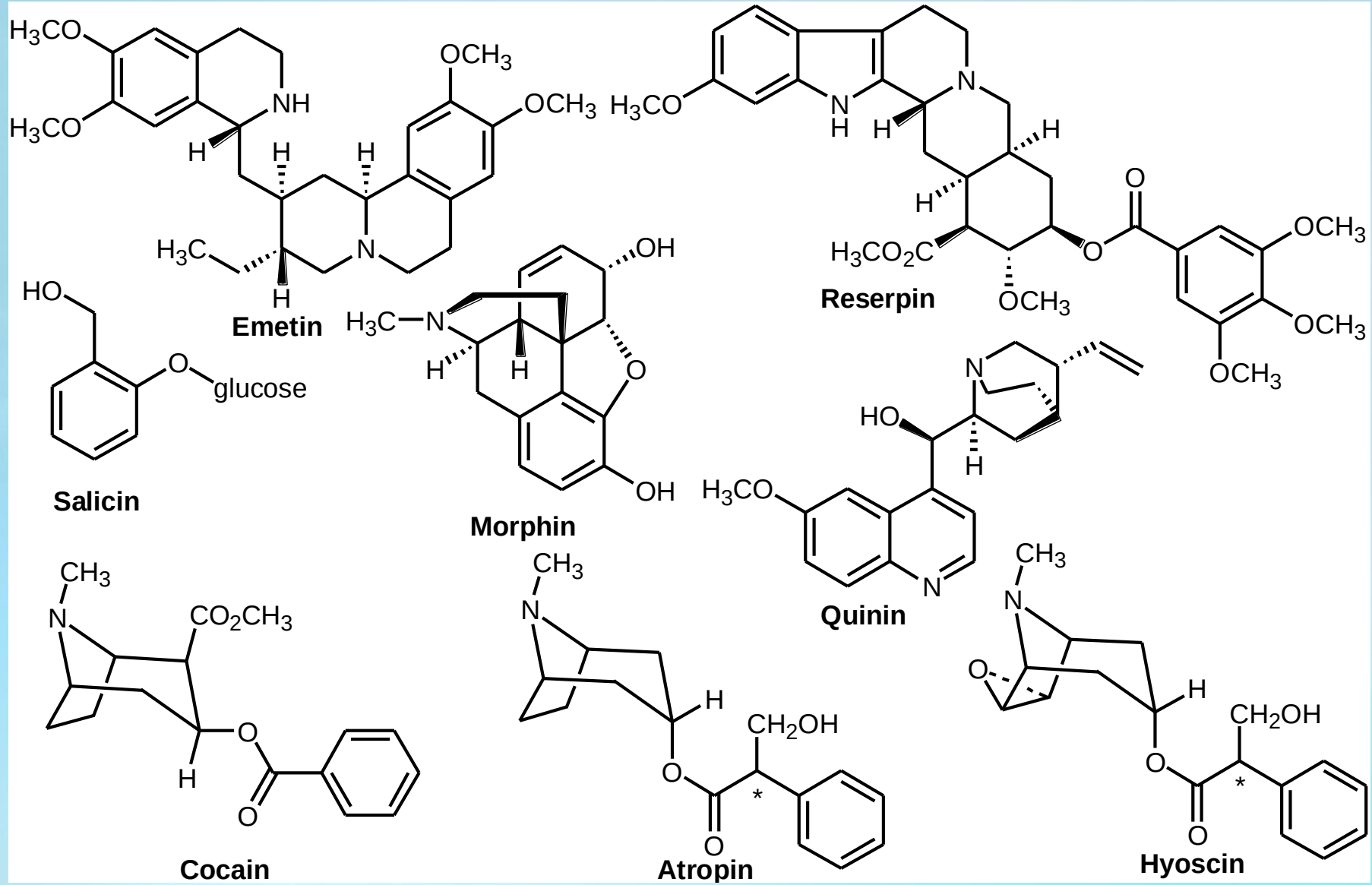
Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro

Teprotid





Long time ago, many active substances were isolated from herbs and plants ➔ Phytochemistry, botanical chemistry

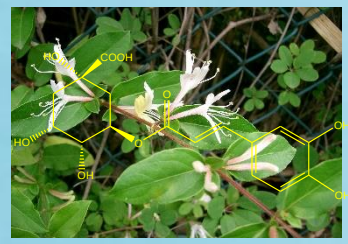


2008 – 2010, NIDQC research collaboration National Research Project

Extraction, isolation, purification and characterization active ingredients from medicinal plants

Prof. Thai Nguyen Hung Thu

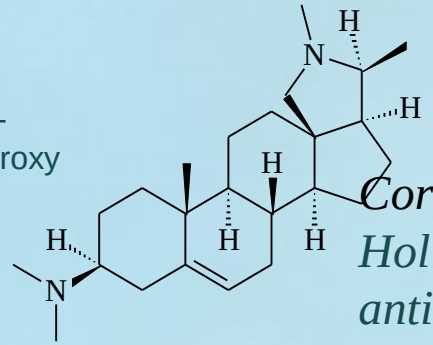
Flos Lonicerae japonicae



Chlorogenic acid

Acid 3-[[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-1,4,5- trihydroxy cyclohexane-carboxylic

$C_{16}H_{18}O_9$; 354.3

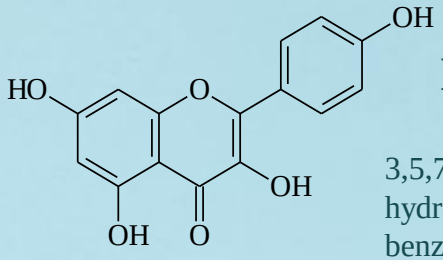


Cortex Hollarenae antidysenterica

Conessin

(3beta)-N,N-dimethylcon-5-enin-3-amine;

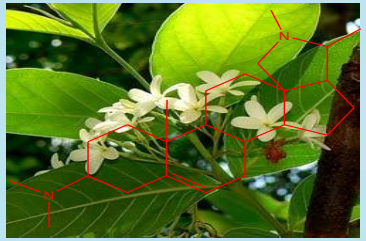
$C_{24}H_{40}N_2$; 56.6

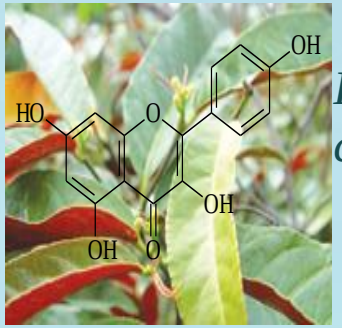


Kaempferol

3,5,7-trihydroxy-2-(4-hydroxyphenyl) -4H-1-benzopyran-4-one;

$C_{15}H_{10}O_6$; 286.2





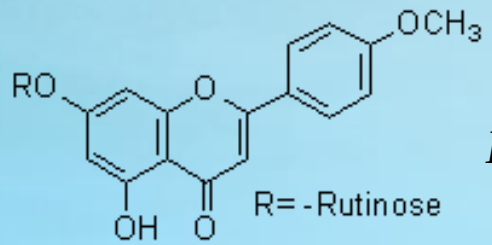
Folium Excoecariae cochinchinesis

Asso.Prof. Trinh Van Lau
Tran Viet Hung, NIDQC

Linarin

Acacetin-7-O-β-D-rutinoside

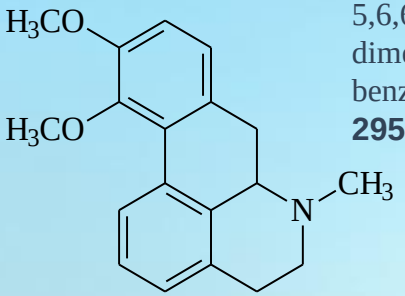
$C_{28}H_{32}O_{14}$, 592.5

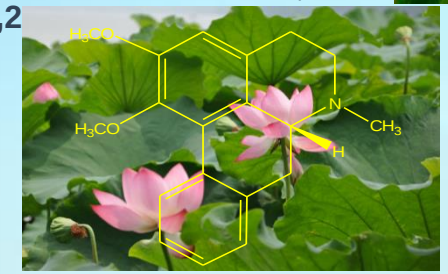


Flos Chrysanthemi indici

Nuciferin

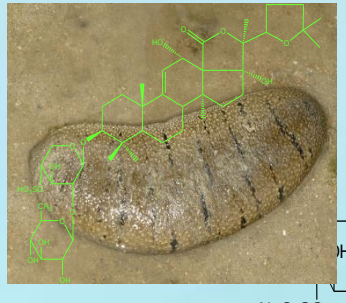
5,6,6a,7 tetrahydro-1,2-dimethoxy-6-methyl-Dia benzoquinolin; **$C_{19}H_{21}NO_2$; 295,2**

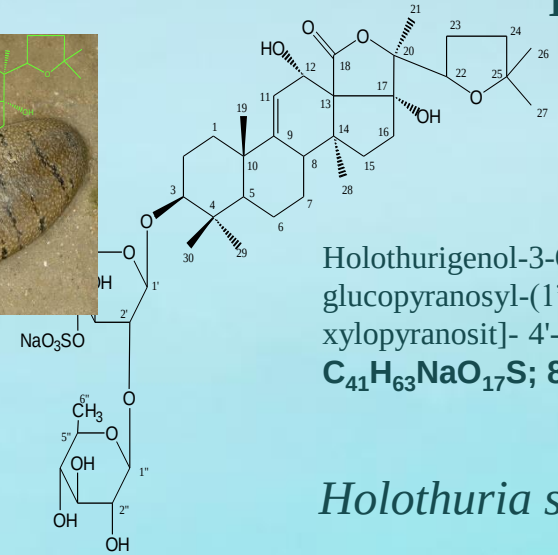




Prof. Chau Van Minh

Holothurin B





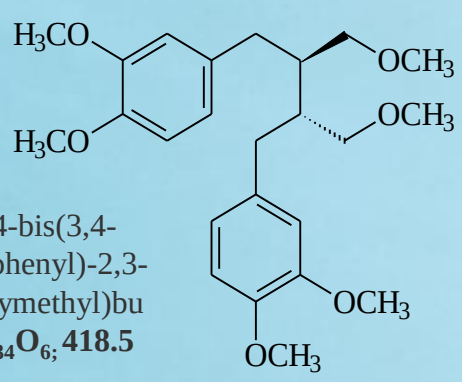
Holothurigenol-3-O-[6”-deoxy-β-D-glucopyranosyl-(1”→2’)-β-D-xylopyranosit]- 4'-O-sulfat natri

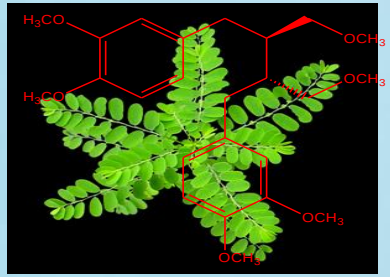
$C_{41}H_{63}NaO_{17}S$; 882.5

Holothuria scabra

Asso.Prof. Le Viet Dung

Phyllanthin

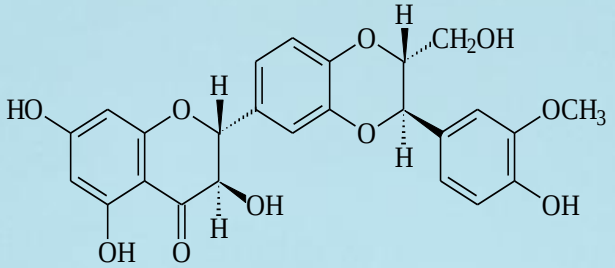


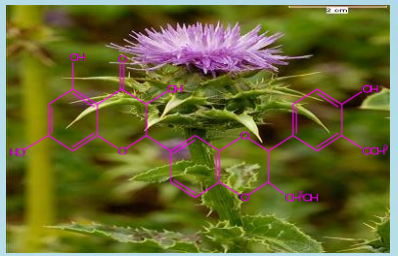


(2R,3R)-1,4-bis(3,4-dimethoxyphenyl)-2,3-bis(methoxymethyl)butane; **$C_{24}H_{34}O_6$; 418.5**

Asso.Prof. Trinh Thi Diep

Silybin

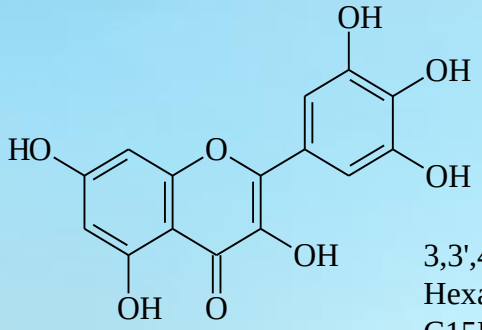


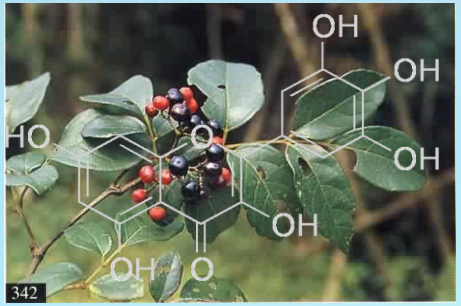


3,5,7-trihydroxy-2-[3-(*R*)-(4-hydroxy-3-methoxyphenyl)-2-(*R*)-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]chroman-4-one); **$C_{25}H_{22}O_{10}$; 482.4**

Prof. Pham Thanh Ky

Myricetin

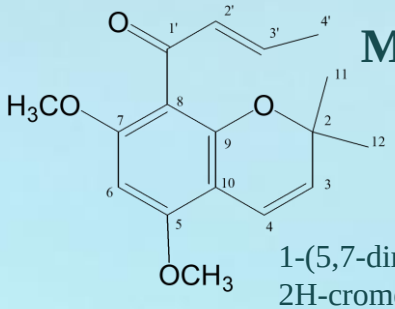


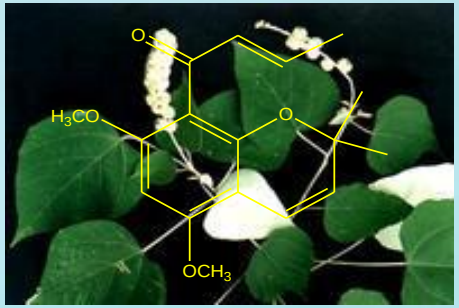


3,3',4',5,5',7-Hexahydroxyflavone
C15H10O8

Prof. Phan Van Kiem

Malloapelta B



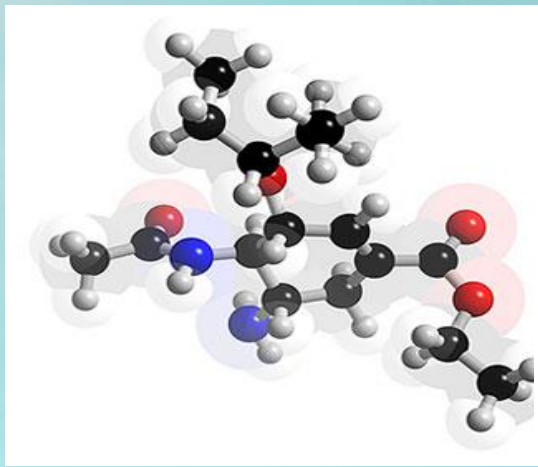


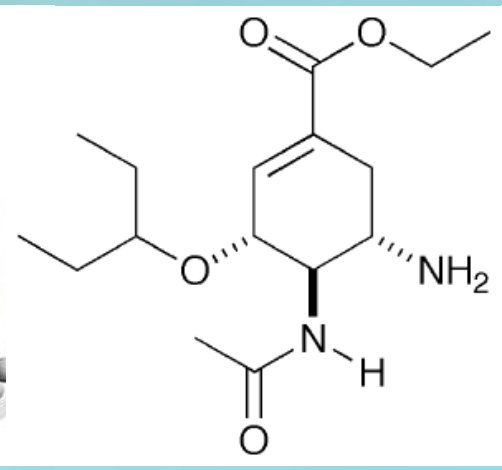
1-(5,7-dimetoxy-2,2-dimetyl-2H-cromen-8-yl)-but-2'-en-1'-on; **$C_{17}H_{20}O_4$; 288.1**

Folium Mallotus apeltae

Bioequivalence (BE) study of generic drugs

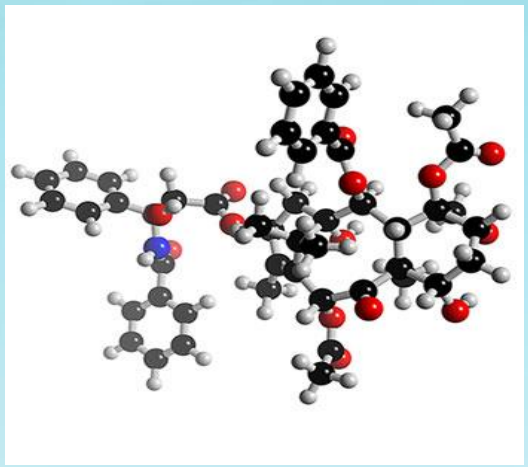
- Patented drug, Invented Drug

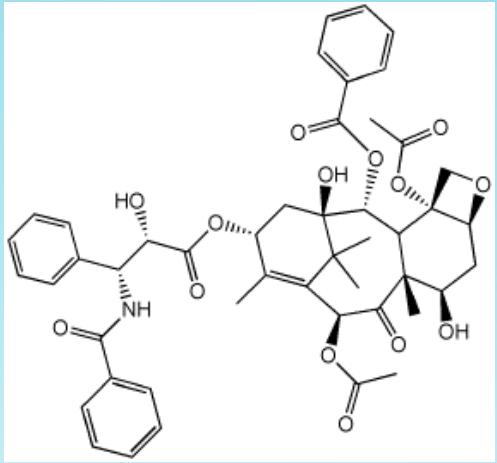




Roche 27/12/1996 (U.S. Patents) 20 years, ➔ 26/12/2016 generic

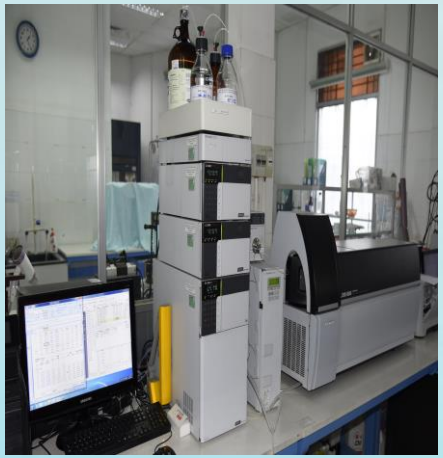
- Generic drug
- Super generic drug (China, India, Brasil)





Bristol-Myers Squibb, 03/03/1992 (U.S. Patents) ➔ 02/03/2012

Analytical method development and validation



U.S. Food and Drug Administration
Protecting and Promoting Public Health
www.fda.gov

Drug Substance Specification

Attribute	Acceptance Criteria (typical values)	Analytical Procedure (for example)
Identity	Matches Standard	IR or HPLC/UV
Assay	98-102%	HPLC
Appearance	Color	Visual
Impurities (Related Substances)	<1% to few %	HPLC
Inorganic Impurities	Heavy Metals (ppm) Na, etc ~ %	Spectroscopy Residue on Ignition
Residual Solvents	ppm to 0.5%	Head-space GC
Particle Size	Case-by-case	Sieve, Laser Diffract.
Solid-State Form	Conforms/limit	Powder X-Ray; IR
Water Content	Few %	Chemical or wgt. loss
Microbial Limits Or Sterility	# of total aerobes and fungi per gram Pathogen (-)	Growth in special media

22

Etablishing Chemical Reference Standards

PCR gene sequencing, DNA identification of medicinal plant, herb medicines

MiniSeq™ của Illumina

KẾT QUẢ ĐỊNH DANH DƯỢC LIỆU

Tên mẫu: Sâm Ngọc linh tươi

Số phân tích: 38G0913

Ngày gửi mẫu: 26/07/2019

Kết quả: Giải trình tự 2 chiều

Tài liệu tham khảo: PMC - Scientific Reports, "DNA based identification of medicinal materials in Chinese patent medicines", Rong Chen,Juan Dong, Xin Cui, Wei Wang, Afshan Yasmeen, Yun Deng, Xiaomao Zeng,and Zhuo Tang.

<F>

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GAT

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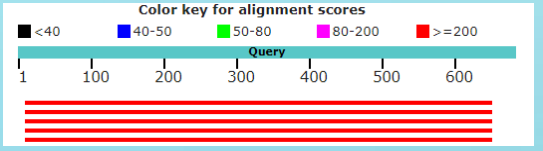
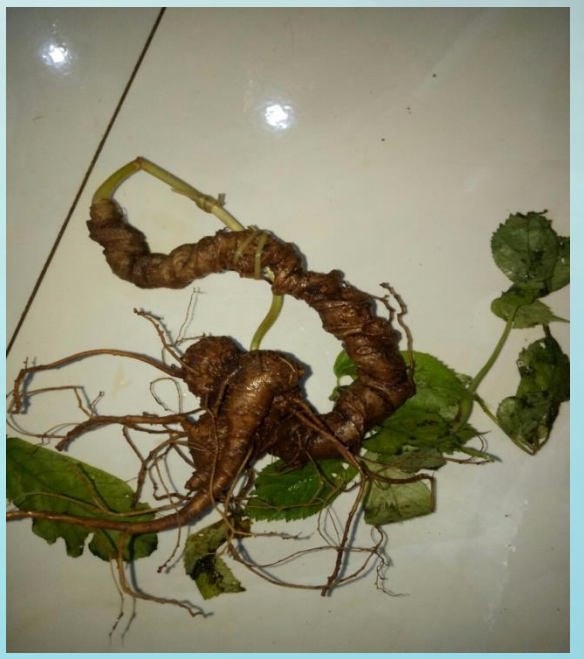
Molecule type	nucleic acid	Program	BLASTN 2.8.1+ ▷Citation
Query Length	678		





Panax vietnamensis

Some other machinery

Supercritical Fluid Extraction

Extraction and evaporation system,

microclimate cabinet

Tabletting machine