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Chemistry

DEVELOPMENT OF A RAPID AND EFFICIENT METHOD FOR QUANTITATIVE DETERMINATION OF N-NITROSODIMETHYLAMINE IMPURITY BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN METFORMIN HYDROCHLORIDE, LOSARTAN POTASSIUM, VALSARTAN AND RANITIDINE MEDICINAL RAW MATERIALS AND ITS PRODUCTS

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In this study, chromatographic conditions were developed for the separation and quantification of N-nitrosodimethylamine (NDMA) by HPLC. Our method would be useful for the rapid screening and quantification of NDMA impurity in Metformin hydrochloride, Losartan potassium, Valsartan and Ranitidine drugs substance and its products.

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***Keywords*:** NDMA, Metformin hydrochloride, Losartan potassium, Valsartan, Ranitidine.

Introduction. In July 2020, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) issued an opinion requiring companies to take measures to limit the presence of nitrosamines in human medicines as far as possible and to ensure levels of these impurities do not exceed set limits [1]. Nitrosamines are classified as probable human carcinogens (substances that could cause cancer) [2]. The limits for nitrosamines in medicines have been set using internationally agreed standards (ICH M7(R1)) based on lifetime exposure. Generally, people should not be exposed to a lifetime risk of cancer exceeding 1 in 100 000 from nitrosamines in their medicines. EU regulators first became aware of nitrosamines in medicines in mid-2018, and took regulatory actions, including recalling medicines and stopping the use of active substances from certain manufacturers.

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A subsequent CHMP review of sartan blood pressure medicines in 2019, led to new requirements for the manufacture of sartans, while its 2020 review of ranitidine recommended an EU-wide suspension of ranitidine medicines. As the toxicity of NDMA is manifested even at $\mu g/kg$ levels, sensitive and specific methods were developed for the determination of NDMA at trace level [3].

NDMA was generated during the tetrazole-formation step owing to the presence of dimethylamine as an impurity or a degradant in N,N-dimethylformamide (DMF) solvent and the presence of nitrous acid generated from sodium nitrite under acidic conditions (Fig. 1).

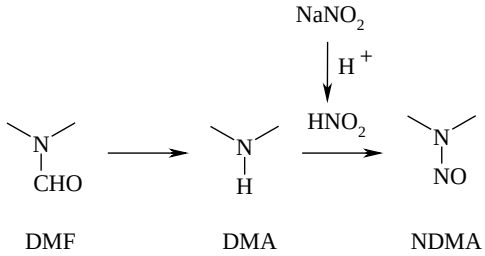

Fig. 1. Prospective mechanism of NDMA production.

Materials and Methods. Standard borosil burettes, pipettes, standard flasks, measuring cylinders and conical flasks are calibrated as per International Conference on Harmonization (ICH) guidelines [4]. Standards and reagents: Metformin hydrochloride (99.9%), Losartan potassium (99.2%), Valsartan (100%), Ranitidine (99.7%) (EDQM), formic acid (Merck), acetonitrile (Merck); methanol (Merck); N-nitrosodimethylamine 200 $\mu g/mL$ in methanol (Merck), water was taken from Milli-Q^R reference water purification system. Equipment: High performance liquid chromatographic (HPLC) system "Shimadzu LC-20-MS" (Japan), automatic injection system (SIL-20A), detector (SPD-M20A), column thermostat (Shimadzu), chromatographic column (Phenomenex C18, 250 $mm \times 5\ \mu m \times 4.6\ mm$), analytical balance ("Shimadzu"), deionized water system ("Purelab", ELGA), "Vortex" key mixer ("Stuart, BioCote", UK), Centrifuge ("Hettich Universal", Germany), 0.45 μm membrane filters ("E-chrom Tech", Taiwan).

General Procedure. About 5 *mg* Metformin hydrochloride, Losartan potassium, Valsartan and Ranitidine were accurately weighed and transferred into a 50 *mL* volumetric flask, 0.5 *mL* (100 μg) NDMA was added, dissolved and diluted with methanol to volume and mixed well.

The drug substance (100 *mg*) or powdered tablet (300 *mg*) was dissolved in 5 *mL* methanol and centrifuged at 6000 *rpm* for 10 *min*. The supernatant was filtered through 0.45 μm membrane filters.

Results and Discussion. However, only a few studies have reported on NDMA analysis using conventional high-performance liquid chromatography (HPLC) [5], especially in drugs. HPLC is the most popular technique for quality control of active pharmaceutical ingredients (API) and products in routine analysis, and it is preferable if NDMA impurity is simultaneously detected with drug substances by a single HPLC analysis. We have developed a gradient elution program using a water-acetonitrile mobile phase containing 0.1% formic acid. The elution gradient used is summarized in Table. The chromatographic separation

was achieved on a Phenomenex C18 column. The column temperature was maintained at 30°C; mobile phase flow rate 1 *mL*/*min*; wavelength 254 *nm*; injection volume of 20 μL. Fig. 2 shows representative chromatograms of a standard mixture of Metformin hydrochloride, NDMA, Losartan potassium, Valsartan and Ranitidine.

Gradient HPLC system of the simultaneous determination of Metformin hydrochloride, NDMA, Losartan potassium, Valsartan and Ranitidine (A (%) – 0.1% formic acid in water; B (%) – 0.1% formic acid in acetonitrile)

Time, *min*	A, %	B, %
0	90	10
6	90	10
9	10	90
13	10	90
15	90	10
20	90	10

Moreover, four samples (Valsartan, Metformin, Losartan potassium, Ranitidine tablet formulations), which had a possibility for NDMA contamination, were analyzed; none of the samples contained NDMA at detectable levels.

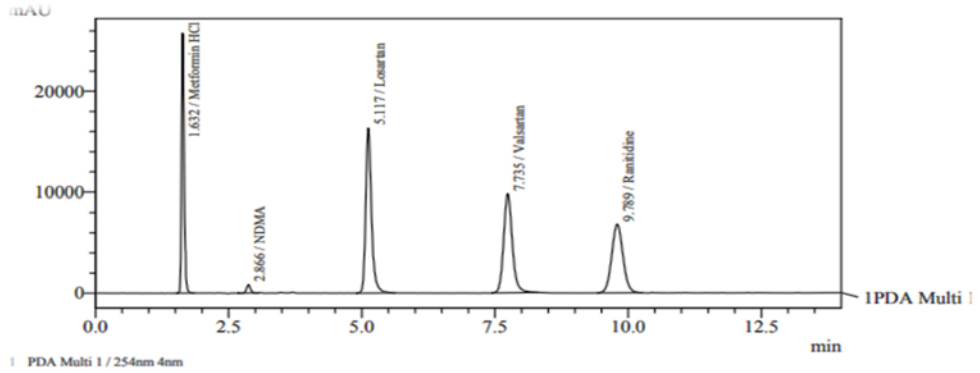

Fig. 2. Representative chromatogram of a standard mixture solution of Metformin hydrochloride, NDMA, Losartan potassium, Valsartan and Ranitidine.

Conclusion. Our method would be useful for the rapid screening and quantification of NDMA impurity in Valsartan, Metformin, Losartan, Ranitidine tablet formulations. In the future, it is planned to validate the quantitative determination of N-dimethylnitrosoamine according to the ICH requirement.

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ՄԵՏՖՈՐՄԻՆ ՀԻԴՐՈՔԼՈՐԻԴԻ, ԼՈԶԱՐՏԱՆ ԿԱԼԻՈՒՄԻ, ՎԱԼՍԱՐՏԱՆԻ ԵՎ ՌԱՆԻՏԻԴԻՆԻ ԴԵՂԱՀՈՒՄՔԵՐՈՒՄ ԵՎ ՊԱՏՐԱՍՏԻ ԴԵՂԱՁԵՎԵՐՈՒՄ ԿՈՂՄՆԱԿԻ ԽԱՌՆՈՒՐԴ N-ՆԻՏՐՈԶՈԴԻՄԵԹԻԼԱՄԻՆԻ ՔԱՆԱԿԱԿԱՆ ՈՐՈՇՄԱՆ ԱՐԱԳ ԵՎ ԱՐԴՅՈՒՆԱՎԵՏ ՄԵԹՈԴԻ ՄՇԱԿՈՒՄ ԲԱՐՁՐ-ԱՐԴՅՈՒՆԱՎԵՏՈՒԹՅԱՆ ՀԵՂՈՒԿԱՅԻՆ ՔՐՈՄԱՏՈԳՐԱՖԻԱՅԻ ԵՂԱՆԱԿՈՎ

Մեր կողմից մշակվել է N-նիտրոզոդիմեթիլամինի բաժանման և քանակական որոշման մեթոդ բարձր արդյունավետության հեղուկային քրոմատոգրաֆիայի եղանակով: Առաջարկված մեթոդը կարող է օգտակար լինել մետֆորմին հիդրոքլորիդի, լոզարտան կալիումի, վալսարտանի և ռանիտիդինի դեղահումքերում և պատրաստի դեղաձևերում կողմնակի խառնուրդ N-նիտրոզոդիմեթիլամինի քանակական որոշման համար:

В. Г. КИРАКОСЯН, С. М. ВАРДАПЕТЯН, Л. А. ВАРДЕРЕСЯН, А. О. ЦАТУРЯН

РАЗРАБОТКА БЫСТРОГО И ЭФФЕКТИВНОГО МЕТОДА КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ПРИМЕСИ N-НИТРОЗОДИМЕТИЛАМИНА В ЛЕКАРСТВЕННОМ СЫРЬЕ ГИДРОХЛОРИДА МЕТФОРМИНА, ЛОЗАРТАНА КАЛИЯ, ВАЛСАРТАНА, РАНИТИДИНА И ИХ ПРОДУКТАХ С ПОМОЩЬЮ ВЫСОКОЭФФЕКТИВНОЙ ЖИДКОСТНОЙ ХРОМАТОГРАФИИ

Нами были разработаны хроматографические условия для разделения и количественного определения N-нитрозодиметиламина с помощью высокоэффективной жидкостной хроматографии. Наш метод может быть полезен для количественного определения примеси N-нитрозодиметиламина в лекарственном сырье метформина гидрохлорида, лозартана калия, валсартана, ранитидина и их продуктах.