U.S. FOOD & DRUG ADMINISTRATION

Development and validation of a RapidFire-MS/MS method for screening of nitrosamine carcinogen impurities N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosodibutylamine (NDBA) and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA) in ARB drugs

Background: Losartan potassium is used to treat high blood pressure. From November 2018 to March 2019, FDA alerted patients and health care professionals to the recall of losartan potassium products by several pharmaceutical companies because of the potential for contamination with carcinogenic nitrosamine impurities, including: (1) N-nitrosodimethylamine (NDMA), (2) N-nitrosodiethylamine (NDEA), (3) N-nitrosoethylisopropylamine (NEIPA), (4) N-nitrosodiisopropylamine (NDIPA), (5) N-nitrosodibutylamine (NDBA) and (6) N-nitroso-N-methyl-4-aminobutyric acid (NMBA). These impurities are believed to have been introduced into the finished products through several pathways that include synthesis and manufacturing routes. OTR has developed an advanced analytics robotics-tandem mass spectrometry method (RapidFire-MS/MS) to screen and quantitate the presence of NDMA/NDEA/NEIPA/NDIPA/NDBA/NMBA nitrosamine impurities in losartan potassium API. The method can be adopted to quantitate these nitrosamine impurities in other "sartan" drug API and products.

Conclusions: A novel RapidFire-MS/MS method has been developed to simultaneously quantify NDMA, NDEA, NEIPA, NDIPA, NDBA and NMBA in losartan potassium API. The method was fully validated according to the ICH Q2R1 guidance Validation of Analytical Procedures and was determined to be ***accurate, precise, specific and linear*** over the corresponding analytical ranges. Detailed validation data was documented in technical report FY19-042-DPQR-T. Below is a table summarizing the LOQ and LOD for all six analytes.

	NDMA	NDEA	NEIPA	NDIPA	NDBA	NMBA
Lower Limit of Quantitation (LOQ), ppm	25	50	0.1	0.25	0.1	0.1
Lower Limit of Detection (LOD), ppm	10	25	0.05	0.1	0.05	0.05

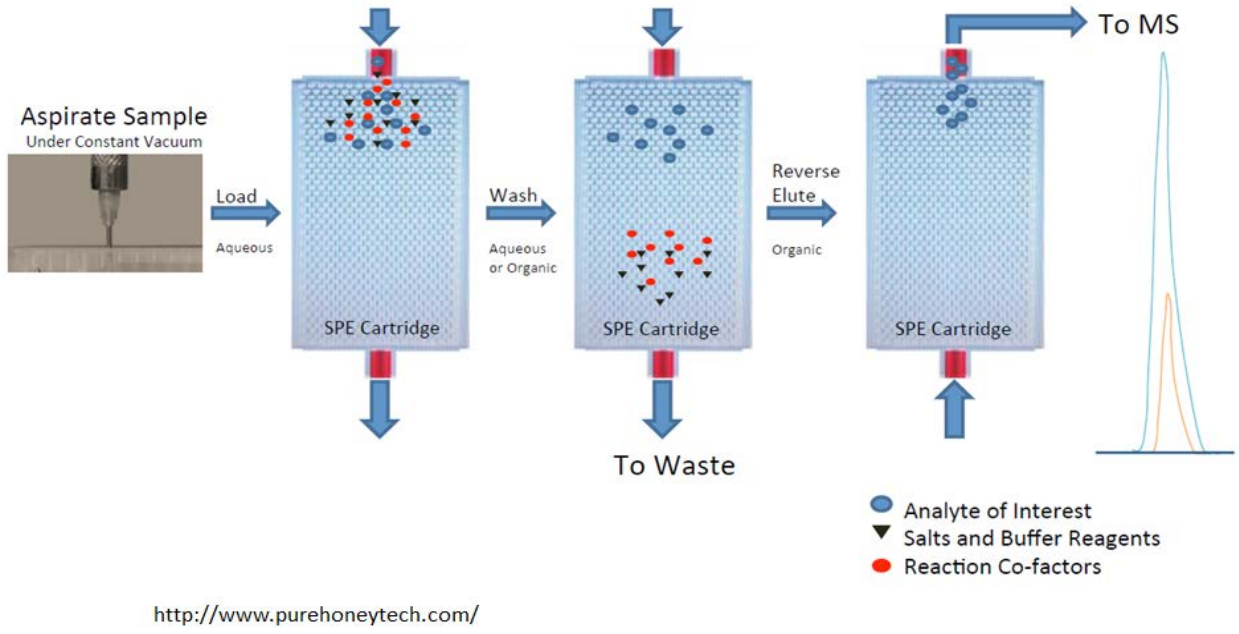
This method would not be sufficient for batch release purposes for verifying that NDMA or NDEA is not present in drugs intended for human use.

NDMA, NDEA, NEIPA, NDIPA, NDBA and NMBA Impurity Assay in Losartan Potassium Drug Substance by RapidFire-MS/MS

Standards

N-nitrosodimethylamine (NDMA): 1 mg/mL in MeOH
N-nitrosodiethylamine (NDEA): 1 mg/mL in MeOH
N-nitrosoethylisopropylamine (NEIPA): 1 mg/mL in MeOH
N-nitrosodiisopropylamine (NDIPA): 1 mg/mL in MeOH
N-nitrosodibutylamine (NDBA): 1 mg/mL in MeOH
N-nitroso-N-methyl-4-aminobutyric acid (NMBA): 1 mg/mL in MeOH
NDEA-*d4*: 1 mg/mL in MeOH
NDBA-*d18*: 1 mg/mL in MeOH
NMBA-*d3*: 1 mg/mL in MeOH

RapidFire-MS/MS workflow



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Sample Preparation and RapidFire methods

Losartan potassium API samples were extracted with 5 volumes of ethyl acetate by sonicating for 30 minutes in a water bath, and then centrifuged at 13000 rpm for 10 minutes. The supernatants were dried under vacuum and then reconstituted with 10x volume of water, and mixed 1:1 with the internal standards solution. Processed samples were then transferred to 96-wells plates. The samples were loaded onto the graphitic carbon SPE cartridge with 0.1% formic acid in water (pump 1, flow rate 1.5 mL/min), and eluted with 0.1% formic acid in methanol (pump 3, flow rate 1 mL/min). The time for aspiration, load/wash, elution and re-equilibration time was 600, 2000, 7000 and 2000 milliseconds, respectively. The turnaround time for each sample was 11.6 seconds.

Mass Spectrometry (Agilent 6460C) source parameters under positive mode.

Parameter	Value (+)	Value (-)
Gas Temp (°C)	250	250
Gas Flow (l/min)	13	13
Nebulizer (psi)	60	60
SheathGasHeater	375	375
SheathGasFlow	11	11
Capillary (V)	1500	0
VCharging	500	0

Calculations:

Peak areas were calculated using RapidFire Integrator® software. A linear calibration model was generated as a weighted (1/Y2) least squares fit of measured peak areas (Y) to known calibration sample concentrations (X) using SAS software. The resulting weighted linear function was used to calculate the concentration of analyte for the study sample or quality control (QC) standard from the assayed peak areas.

Data acquisition parameters on the QQQ Mass Spectrometer in positive mode

Analyte	Precursor ion m/z	Product ion m/z	Fragmentor	CE	Cell accelarator voltage	Dwell time (ms)	Polarity
NDBA-*d18* , Quantifier	177.2	113.1	30	15	3	2	Positive
NDBA-*d18* , Qualifier	177.2	66.3	30	15	3	2	Positive
NDBA, Quantifier	159.1	103.1	30	10	3	2	Positive
NDBA, Qualifier	159.1	57.2	30	10	3	2	Positive
NMBA-*d3* , Qualifier	150.2	87.1	40	10	3	2	Positive
NMBA-*d3* , Quantifier	150.2	47.2	40	10	3	2	Positive
NMBA, Qualifier	147.1	117	40	10	2	20	Positive
NMBA, Quantifier	147.1	44.2	40	10	3	20	Positive
NMBA-*d3* , Quantifier	148.2	62.1	90	10	2	2	Negative
NMBA-*d3,* Qualifier	148.2	42.2	90	20	2	2	Negative
NMBA, Quantifier	145.1	59.2	90	9	2	20	Negative
NMBA, Qualifier	145.1	41.3	90	17	2	20	Negative
NDIPA, Quantifier	131.1	89.1	40	10	2	4	Positive
NDIPA, Qualifier	131.1	47.2	40	20	3	4	Positive
NDIPA, Qualifier	131.1	41.2	40	20	3	4	Positive
NEIPA, Quantifier	117.1	75.2	60	7	3	4	Positive
NEIPA, Qualifier	117.1	47.2	60	14	3	4	Positive
NDEA-*d4* , Quantifier	107.1	77.3	30	10	2	2	Positive
NDEA-*d4* , Qualifier	107.1	47.3	30	16	2	2	Positive
NDEA, Qualifier	103.1	103.1	30	3	0	4	Positive
NDEA, Quantifier	103.1	75	30	11	1	10	Positive
NDEA, Qualifier	103.1	47	30	15	1	10	Positive
NDMA, Qualifier	75.1	58	30	8	2	4	Positive
NDMA, Quantifier	75.1	43	30	21	1	4	Positive

Example Chromatogram of Individual Analytes Demonstrating Method Specificity