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Development of dispersive inclusion complex microextraction for the analysis of nitrosamines in medicinal products

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ABSTRACT

A new dispersive inclusion complex microextraction (DICM) approach coupled with ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) for the determination of n-nitrosamine impurities in different medicinal products is demonstrated for the first time. The proposed DICM procedures consist of a dispersive liquid phase microextraction steps employing cyclodextrin as an inclusion complex agent to extract n-nitrosamines namely N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodiisopropylamine (NDIPA), N-ethyl-N-nitrosoisopropylamine (NEIPA) and N-nitroso-di-n-butylamine (NDBA) present in the medicinal products. The sample solutions were prepared by mixing 5% (m/v) NaCl solution with 1.5 mM β-cyclodextrin and 20 mM sodium dodecyl sulphate to form a stable inclusion complex and subsequently extracted into dichloromethane as an extraction solvent. The enriched solution was reconstituted into aqueous solution prior to UPLC-MS/MS analysis. The method showed good linearity in the range of 0.036–1 ng/mL with a correlation coefficient of at least 0.995, acceptable reproducibility (RSD 0.5–5.8%, n=5), low limits of detection (0.011–0.018 ng/mL), and satisfactory relative recoveries (96–105%). The results obtained were found to be at least 10-fold more sensitive comparable to those obtained using validated direct sample dissolutions coupled with UPLC-MS/MS approach.

1. Introduction

N-nitrosamines are organic compounds containing nitroso group with a chemical structure of (R1N(-R2)-N=O), which can be found in drinking water, cooked meat, dairy products, pharmaceutical products, and formed endogenously [1–3]. Since 2018, a nitrosamine impurity, namely N-nitrosodimethylamine (NDMA), was detected in pharmaceutical drugs namely angiotensin II receptor blockers (ARBs) i.e. valsartan, losartan, and irbersartan, ranitidine, and metformin, which resulted in multiple voluntary product recalls worldwide [4]. The majority of N-nitrosamines are mutagenic carcinogens described as a cohort of concern (CoC) and classified by the International Agency for Research on Cancer (IARC) as probable human carcinogens which are deemed to be a health hazard. Generally, N-nitrosamines are bioactivated to reactive intermediates, which might interact with cellular targets in various organs and tissues, causing potential DNA damage.

The USFDA has recommended the interim daily acceptable intake (AI) limits for the N-nitrosamine impurities, which are 96 ng/day for NDMA and 26.5 ng/day for the N-nitrosodiethylamine (NDEA), N-nitrosodiisopropylamine (NDIPA), N-ethyl-N-nitrosoisopropylamine (NEIPA) and N-nitroso-di-n-butylamine (NDBA), as shown in Table S1 [5]. The acceptable maximum daily intake limit of an N-nitrosamine impurity by a patient is calculated by dividing the acceptable daily intake limit against the maximum daily dose for a particular drug product. Hence, sensitive methods with limits of quantitation (LOQ) in the ppb range are needed to meet the low AIs recommended for nitrosamines.

The United States Pharmacopeia (USP) has recently proposed analytical testing procedures for the monitoring of N-nitrosamine impurities in medicinal products pursuant to USP General Chapter 1469 [6]. The detection limits down to ppb level could be achieved by employing GC-MS/MS for volatile N-nitrosamines and LC-HRMS as well as LC-MS/MS for both volatile and non-volatile N-nitrosamines. Nevertheless, a sample pretreatment step with satisfactory clean-up and preconcentration adequacy is essential

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to ensure the method LOQ is as low as reasonably practical in particular for medicinal products with a high maximum daily dosage (> 2 g of active pharmaceutical ingredient per day) [7].

Several methods have been demonstrated for the determination of N-nitrosamine impurities in pharmaceutical products since the recall of ARBs drug. Most of the reported approaches employed the direct injection of the samples into the chromatographic system with tandem mass spectrometry for quantification [5,8]. Several chromatographic techniques such as gas chromatography (GC) and liquid chromatography (LC) have been used in conjunction with sample pretreatment steps. Common sample pretreatment approaches are, for example, liquid-liquid extraction, solid phase extraction, liquid-phase microextraction and dispersive liquid phase microextraction [9,10]. The major drawback of the reported sample pretreatment approaches is the extraction principles are greatly dependent on the partition coefficient of the N-nitrosamines, which leads to unsatisfactory extraction recovery especially on less hydrophobic targeted analytes such as the NDMA and NDEA.

Dispersive liquid-liquid microextraction (DLLME) has become an attractive sample pretreatment technique due to its simplicity, cost effective, and satisfactory extraction recovery performance [11]. Nevertheless, the conventional DLLME is favorable for the extraction of mid and highly hydrophobic targeted compounds. A series of applications of the method for N-nitrosamines analysis of diverse sample matrices have been described [12–21]. Gimenez-Campillo and coworkers [22] demonstrated the use of DLLME to monitor several N-nitrosamines present in the drug products. Nonetheless, NEIPA and NDIPA which were listed in USFDA guidance were not included. Moreover, the limit of quantifications achieved were found not meeting the desired quantification limits set by the regulatory authority. A comprehensive approach for the determination of N-nitrosamine impurities in pharmaceutical drug products at sub-ppb level has yet to be discussed.

In this study, dispersive inclusion complex microextraction (DICM) that combines the concept of DLLME and inclusion complex, was proposed. This features inclusion complex traps analyte based on the chemistry of host-guest interaction instead of polarity. A series of applications of inclusion complex for analysis in different samples have been reported [23–29]. The DICM protocol, coupled with UPLC-MS/MS analysis, was employed for the determination of five n-nitrosamine impurities in pharmaceutical drug samples in this study. This research aims to further enhance the overall method sensitivity against the existing N-nitrosamine testing procedure recommended in USP General Chapter 1469. We will demonstrate an alternative to dispersive liquid phase microextraction, termed as the DICM approach, coupled with ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) for the quantification of N-nitrosamines in selected medicinal products.

2. Experimental

2.1. Reagents and materials

N-nitrosamines namely NDMA, NDEA, NEIPA, NDIPA and NDBA were purchase from Toronto Research Chemicals (TRC, Ontario, Canada). α-Cyclodextrin (α-CD), β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) were purchased from Tokyo Chemical Industry (TCI, Tokyo, Japan). Sodium dodecyl sulfate, sodium chloride, dichloromethane (DCM), chloroform ($CHCl_3$), methyl-tert-butyl ether (MtBE), acetonitrile (MeCN), acetone (Ace), ethanol (EtOH) and methanol (MeOH) were obtained from Merck (Damstadt, Germany). Ultrapure deionized water was produced from a Millipore Direct-Q3 system (Merck Millipore, Damstadt, Germany). The mobile phases were prepared daily. Standard stock solutions of N-nitrosamines mixture at concentration of 100 µg/mL were prepared in methanol and kept at 4 °C. Working standard solutions of lower concentrations were prepared by dilution with deionized water. Medicinal products namely Valsartan tablet, Losartan tablet and Ranitidine injectable ampoule were obtained from the University Health Center located in UTM Johor Bahru, Malaysia. Other reagents and chemicals used were at least of analytical grade and used without any further purification.

2.2. Direct sample dissolution procedure

The following direct sample dissolution procedure was adapted from USP General Chapter 1469 procedure. For valsartan and losartan tablets, five tablets were weighed and ground into powder. An amount of the tablet powder, equivalent to 100 mg of active pharmaceutical ingredient (API), was accurately weighed and transferred to a 15 mL centrifuge tube. The sample powder was dissolved with 10 mL of aqueous solution containing 20% MeOH. The mixed solution was vortexed until homogeneous, followed by centrifugation for 10 min at 5000 rpm. The supernatant was collected and filtered using 0.22 µm PVDF syringe filter prior to UPLC-MS/MS analysis. For Ranitidine injectable liquid solution, 2 ampoules equivalent to 100 mg of API were transferred into a 15 mL centrifuge tube and further diluted to 10 mL using 100% deionised water. The mixed solution was vortexed for 2 min until homogeneous and filtered using 0.22 µm PVDF syringe filter prior to UPLC-MS/MS analysis.

2.3. Dispersive inclusion complex microextraction (DICM) procedure

For valsartan and losartan tablets, five tablets were weighed and ground into powder. An amount of the tablet powder, equivalent to 100 mg of active pharmaceutical ingredient (API), was accurately weighed and transferred to a 15 mL centrifuge tube (as shown in Fig. S1). The sample powder was dissolved with 10 mL of diluent, which consisted 5% (m/v) NaCl, 1.5 mM β-CD and 20 mM SDS in 20% (v/v) MeOH solution. The mixed solution was vortexed until homogeneous, followed by centrifugation for 10 min at 5000 rpm. The supernatant was collected and filtered using 0.22 µm PVDF syringe filter. For Ranitidine injectable liquid solution, 2 ampoules equivalent to 100 mg of API were transferred into a 15 mL centrifuge tube and further diluted to 10 mL using diluent containing 5% (m/v) NaCl, 1.5 mM β-CD and 20 mM SDS in 100% water. The mixed solution was vortexed for 2 minutes until homogeneous. 1 mL of supernatant collected from valsartan and losartan solution or 1 mL of ranitidine mixed solution was then transferred into a 2 mL microcentrifuge tube and 300 µL of DCM was then added to initiate the liquid phase microextraction process, vortexed for 30 s, and centrifuged for 5 min at 14000 rpm. A total of 200 µL of enriched extractant was withdrawn using a 100 µL microsyringe and transferred to a 250 µL glass insert for HPLC vial. Finally, the solution was evaporated with a stream of nitrogen gas and reconstituted with 20 µL of DI water (for valsartan and losartan) or 50% (v/v) MeOH (for ranitidine) for UPLC-MS/MS analysis. Note that the evaporation step should be conducted only with a gentle flow of nitrogen gas to ensure good repeatability of the results.

2.4. UPLC-MS/MS analysis

The treated sample solutions were injected onto a Waters ACQUITY UPLC H-Class liquid chromatography system coupled with a Xevo TQ-S Micro Triple Quadruple Mass Spectrometer (MS) equipped with an atmospheric-pressure chemical ionization (APCI) source (Waters Analytical Instruments, United States), and operated in multiple reaction monitoring (MRM) mode. The column used was Waters Acquity UPLC® HSS T3, 100 × 2.1 mm, i.d. 1.8 µm

(Waters Analytical Instruments, United States). The mobile phase A and B were 0.1% formic acid with 2 mM ammonium formate in water and 0.1% formic acid with 2 mM ammonium formate in methanol, respectively. The column temperature was maintained at 40°C, autosampler temperature was set at 10 °C and injection volume was fixed at 5 µL. Chromatographic separation was performed using the gradient elution as follows: 95% A, at 0–0.24 min; 90% A, at 0.54 min; 80% A, at 1.54 min; 70% A, at 2.04 min; 20% A, at 6.50 min; 10% A, at 7.00 min; 2% A, at 7.50 min; 95% A, at 7.60–11.00 min. The MS system was operated using APCI in positive-ion mode. Other conditions were ion spray voltage (20 V), desolvation gas flow (300 L/hr), heating gas flow (100 L/hr), drying gas flow (100 L/hr), APCI probe temperature (300 °C), and source temperature (150 °C). The conditions of N-nitrosamines multiple reaction monitoring (MRM) transitions are demonstrated in Table S2.

3. Results and discussion

3.1. Optimization of the dispersive inclusion complex microextraction

In this study, the new proposed dispersive inclusion complex microextraction (DICM) allows for efficient extraction of N-nitrosamines with a wider range of hydrophobicity. The sample solution containing targeted analytes was introduced with cyclodextrin to form an inclusion complex and subsequently extracted into an organic solvent before being rapidly injected into an aqueous sample containing inclusion complex with analyte. Subsequently, a phase separation is performed, and the enriched analyte can then be determined in the organic phase. Several factors affecting the extraction efficiency of DICM were comprehensively examined to seek optimum conditions.

3.1.1. Selection of extraction and dispersive solvents

An ideal extractant in conventional dispersive type of liquid phase microextraction should demonstrate characteristics such as high extraction capability for analytes of interest, low solubility in water, and low volatility. On the other hand, dispersant solvent is commonly added mainly to enlarge the contact area between the extractant and the sample solution, which could significantly enhance the extraction efficiency. Hence, three extractants, namely dichloromethane (DCM), chloroform ($CHCl_3$) and methyl-tert-butyl ether (MtBE), were examined in combination without/with dispersants, i.e., acetonitrile (MeCN), methanol (MeOH), acetone (Ace) and ethanol (EtOH). The selection of extractants was first performed by using ethanol as dispersant solvent. It was found that since MtBE has lower density than the sample solution, the analyte enriched solvent was hardly collected. The use of $CHCl_3$ and DCM led to a clear phase separation after centrifugation. Nevertheless, the recoveries of N-nitrosamines were comparably lower when $CHCl_3$ was used. Therefore, DCM was selected as an extractant and its performance both with and without various dispersants was evaluated. In order to maintain consistency, 300 µL of each dispersant with 300 µL of DCM was added to the 1 mL of the sample solution in all cases studied. As can be seen in Fig. 1, the optimum extraction recoveries were obtained when no dispersant was employed. Note that the proposed DICM, unlike the conventional DLLME, does not require the dispersive solvent to facilitate the liquid phase microextraction process. Hence, the extraction procedure was further examined without the use of dispersant and DCM was selected as the extractant.

3.1.2. Effect of cyclodextrin and sodium dodecyl sulfate

In the newly proposed DICM approach, it is hypothesized that the mechanism involves forming of an N-nitrosamine/cyclodextrin inclusion complex followed by extraction into the DCM organic phase, rather than through partitioning of targeted analytes into organic droplets, which is the case in DLLME. It is also predicted that the addition of inclusion complex agent such as cyclodextrin, will significantly enhance the overall extraction efficiency. The effects of the addition of various types of cyclodextrin in the sample solution with and without SDS as an additive, was shown in Fig. 2a. It can be seen that the adsorption of targeted analyte by CD is highly dependent on the stability of the inclusion complex formed, whereby the closer fit between the host CD and guest N-nitrosamines, the higher the stability of the inclusion complex formed [30]. Generally, the cavity size of cyclodextrin is increasing from α-CD, β-CD and γ-CD. Based on the data shown in Fig. 2a, a similar extraction performance was noted for sample solutions without CD/SDS and with α-CD. This might be due to no trapping effect against N-nitrosamines with the use of α-CD due to its small cavity size. A slight improvement on the extraction efficiency was observed when β-CD and γ-CD were added. This is probably

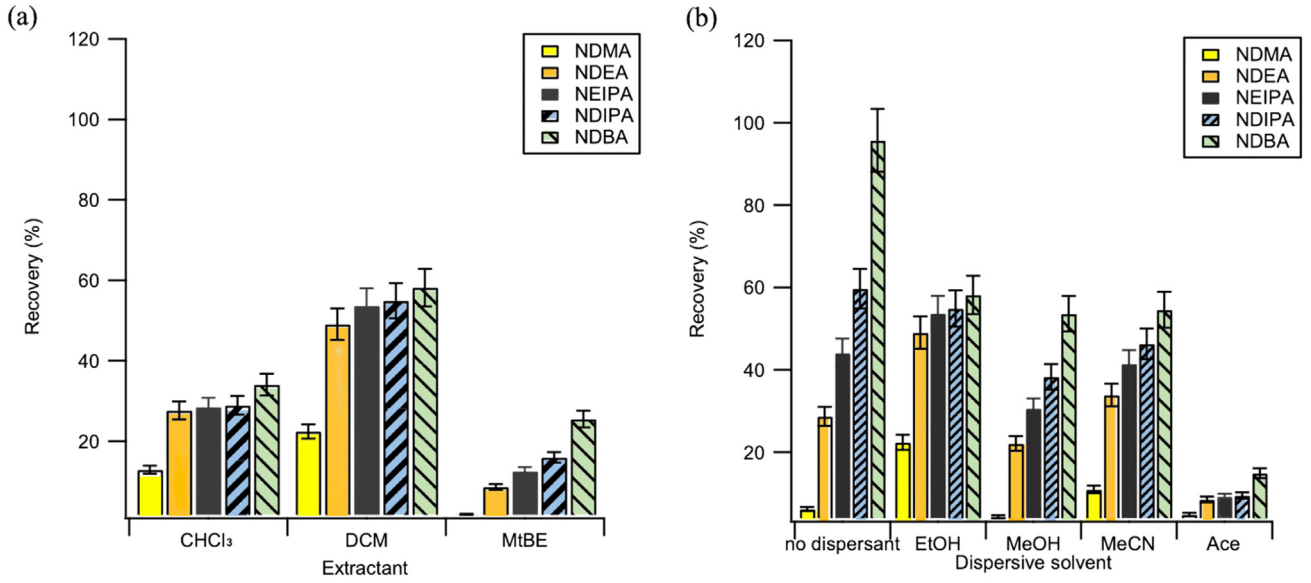

Fig. 1. Effect of (a) extractant and (b) dispersive solvent on the extraction efficiency of N-nitrosamines. Extraction conditions: sample volume, 1 mL; extractant volume, 300 µL; dispersant solvent volume, 300 µL; sample pH, 6.0; concentration of each N-nitrosamines, 0.1 ng/mL. (Error bars represent standard deviations of results, $n = 3$).

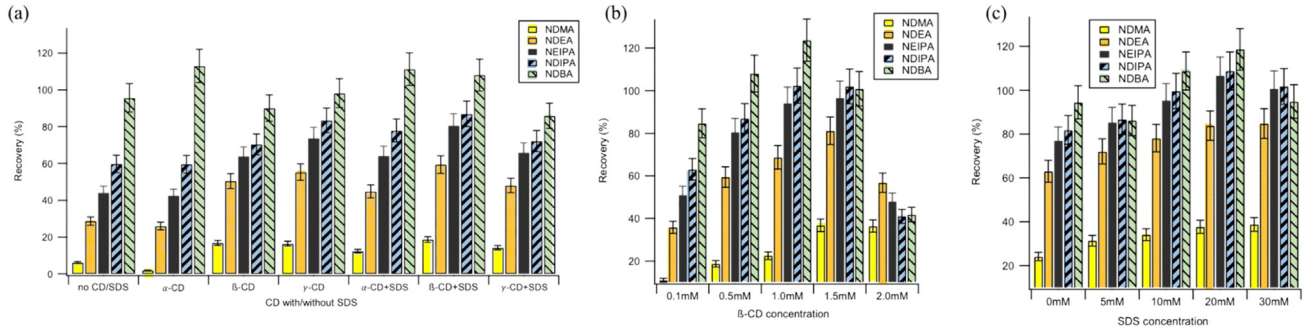

Fig. 2. Effect of (a) CD with/without SDS, (b) β-CD concentration, and (c) SDS concentration on the extraction efficiency of N-nitrosamines. Extraction conditions: sample volume, 1 mL; extractant volume, 300 µL; sample pH, 6.0; concentration of each N-nitrosamines, 0.1 ng/mL. (Error bars represent standard deviations of results, $n = 3$).

due to the better fit between the cavity of β-CD and γ-CD and N-nitrosamines which enhance the extraction performance [31]. On the other hand, it can be noted that the addition of SDS as an additive together with each individual CD had further enhanced the extraction recovery especially on the less hydrophobic NDMA and NDEA. SDS in this case could behave as an emulsifier during DICM which reduces the interfacial tension between the two immiscible solutions, resulting in enlarging the contact area between targeted analytes and DCM [32]. Among the examined combinations, β-CD with SDS was found to be capable of providing the highest extraction efficiency. The β-CD cavity with a diameter of approximately 0.60–0.65 nm and height of 0.78 nm has been proven to be the most suitable host among the three CDs to form inclusion complex with most of the drugs [33]. This approach should also be expected for other small organic molecules present in diverse sample matrices.

The effect of β-CD and SDS concentrations against the DICM extraction efficiency were further investigated by first evaluating the β-CD concentration in the range of 0.1–2.0 mM. As illustrated in Fig. 2b, the extraction rate gradually increases from 0.1 mM and reached the optimal when 1.5 mM of β-CD was added to the samples. Further increase of β-CD to 2.0 mM resulted in a decrease of extraction efficiency, which might be due to the aggregation of CD when the level used exceeded the critical aggregation concentration of 1.6 mM [34]. The effect of the SDS concentration was further evaluated in the range of 0–30 mM. As shown in Fig. 2c, a significant improvement in the rate of extraction was observed when the SDS concentration was increased from 0 to 20 mM and then reached a maximum range of 20–30 mM. Based on the results obtained, β-CD and SDS were fixed at a level of 2.0 mM and 20 mM respectively for the subsequent experiments.

3.1.3. Effect on sample volume, salt, and sample pH

Different volumes of sample solution to extractant were studied to seek for the optimum extraction conditions. Note that the extractant volume was fixed at 300 µL and sample volume of 500, 1000, 2000, 3000, and 4000 µL were tested for the optimum performance. A significant increase in extraction recovery was observed when sample volume was increased from 500 µL and reached it is optimum performance at 1000 µL. The extraction efficiency was found to gradually decrease when larger sample volumes (2000–4000 µL) were applied in the extraction. This phenomenon might be due to the saturation of the DCM extractant for a large sample volume. Hence, the sample volume to extractant of 10:3 was adopted.

The addition of salt to the sample solution has been prevent able to decrease the solubility of the analytes in particular less hydrophobic compounds such as NDMA and NDEA, and therefore enhance the extraction due to the salting out effect. The effect of adding sodium chloride at concentration range from 0 to 10% (w/v) was investigated. The extraction efficiency for N-nitrosamines obtained optimum values when 5% (w/v) of sodium chloride was added to the samples. On the other hand, targeted N-nitrosamines examined in this work are impurities that having pKa values in the range of approximately 3.1–3.3. Hence, these analytes are present in the entirely neutral state at the pH level 6 and above. Hence, the sample solution with pH 6 and above was not expected to have a significant impact on the extraction efficiency. The effect of the sample pH was investigated in the range of pH 2–10, indeed, the extraction efficiency gradually increased from 2 to 6 and no significant differences in terms of extraction efficiency were observed from pH 6 to 10 (results not shown). Therefore, sample solutions with a pH value of 6.0 were used in the subsequent experiments.

3.2. Method validation

The optimum DICM parameters were as follows: 1 mL of sample solution at pH 6 containing 5% (m/v) NaCl, 1.5 mM of β-CD, and 20 mM of SDS. The sample solution is extracted into 300 µL of DCM as an extractant. Other experimental steps were previously described in Section 2.3. A series of experiments to determine linearity, limits of detection (LODs), limits of quantification (LOQs), repeatability, and sensitivity enhancement factor [35] were performed for direct sample dissolution prior to UPLC-MS/MS (Section 2.2) and newly established DICM coupled with UPLC-MS/MS approach, respectively. The results based on a method for Sartan drug and Ranitidine Injectable are given in Table 1. Calibration curves for DICM-UPLC-MS/MS approach were acquired for standard solutions at eight concentration levels in the range from 0.036 to 1 ng/mL, using optimized extraction conditions. The curves of the peak area versus analyte concentration (ng/mL) were found to be linear for this range with good correlation coefficients of at least 0.9950. The LOD values for the N-nitrosamines, which are in the range of 0.011–0.018 ng/mL, were found to be approximately 11–18 fold better than the direct sample dissolution-UPLC-MS/MS approach. The precision of the method was satisfactory with the relative standard deviation (RSD) being lower than 6%. The intraday and interday variabilities for analyte peak intensities were found to be in the range of 0.5–5.5% and 5.1–8.4%, respectively (RSD). Typical chromatograms of a drug samples and drug samples spiked with N-nitrosamines are shown in Fig. 3. It can be seen that no interfering peaks were found in the extracted blank drug samples under the multiple reaction monitoring detection mode. The method recovery was evaluated by determining the concentrations of the analytes spiked at three different concentrations (0.1, 0.5, and 1.0 ng/mL). The concentrations were obtained by comparison with the calibration curves for peak areas

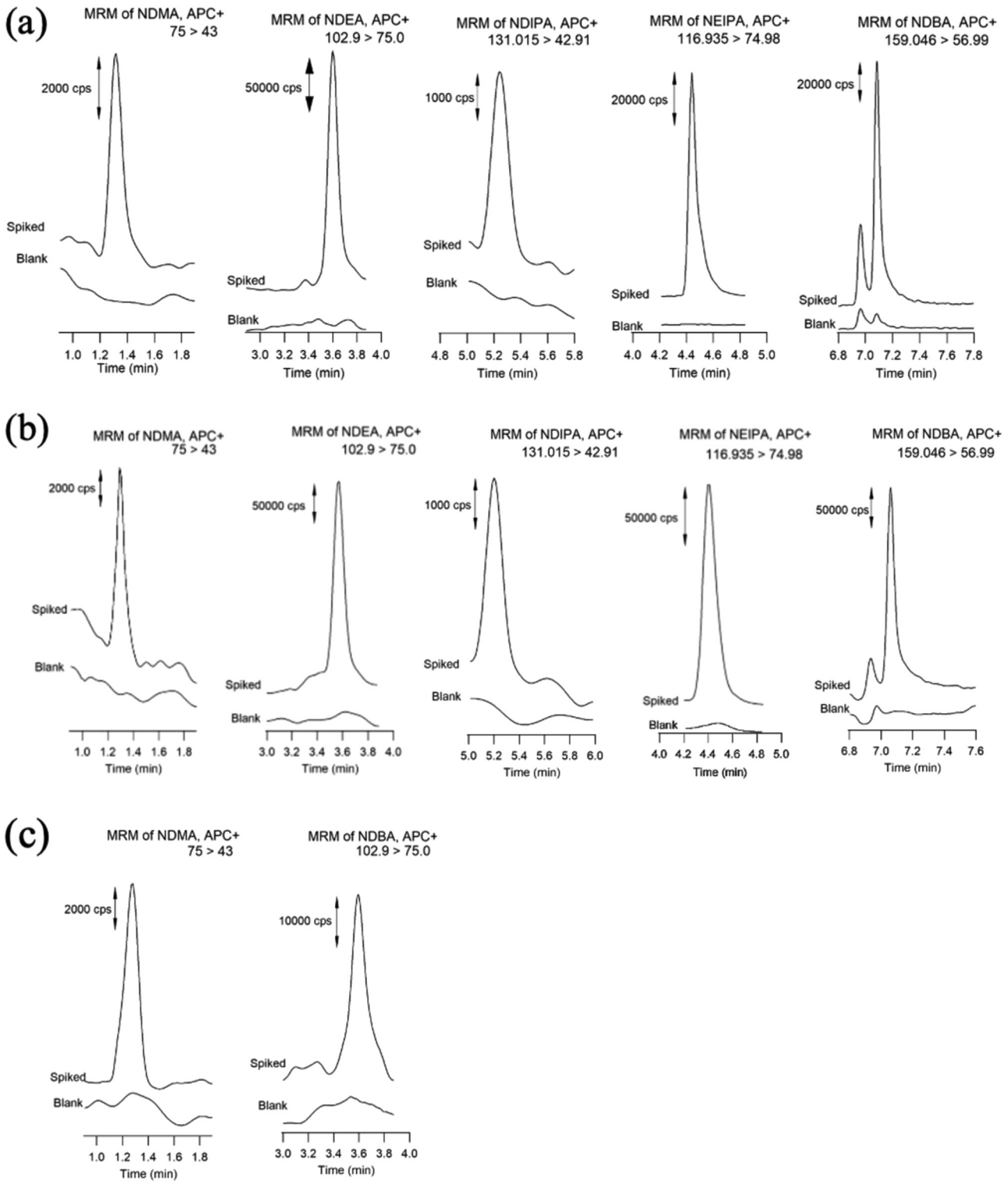

Fig. 3. Chromatograms for the separation of N-nitrosamines in blank and spiked (a) Valsartan tablet, (b) Losartan tablet and (c) Ranitidine injectable samples after the DICM-UPLC-MS/MS. Extraction conditions: sample volume, 1 mL; extractant volume, 300 µL; sample pH, 6.0; concentration of each N-nitrosamines, 0.1 ng/mL. Peaks: (1) NDMA, (2) NDEA, (3) NEIPA, (4) NDIPA, (5) NDBA.

obtained with standards that had also been subjected to the extraction procedure. This compensates for the different degrees of extraction efficiency for the different analyte species. The results, summarized in Table 2, are certainly acceptable and demonstrate that the extraction efficiencies are stable and not affected by the background matrices of the medicinal products.

3.3. Analysis of commercial medicinal products

A total of three medicinal product samples which includes Valsartan tablet, Losartan tablet and Ranitidine injectable were obtained from Health Center of Universiti Teknologi Malaysia, Johor, Malaysia. The drug samples were analyzed for N-nitrosamines ei-

Table 1
Linear range, regression data, limits of detection (LODs), limits of quantitation (LOQs), method repeatability, and sensitivity enhancement factors for five n-nitrosamine impurities in spiked medicinal products.

Analytes	Linearity (ng/mL)	Correlation of determination (r^2)	LOD[a] (ng/mL)	LOQ[b] (ng/mL)	Repeatability[c] Intraday (n=5)	%RSD Interday (n=5, 3 days)	SEF[d]
Direct sample dissolution – UPLC-MS/MS							
Valsartan tablet							
NDMA	0.500-1.500	0.9993	0.200	0.500	1.4	2.5	-
NDEA	0.500-1.500	0.9995	0.200	0.500	0.8	0.8	-
NEIPA	0.300-1.500	0.9992	0.100	0.300	1.0	1.3	-
NDIPA	0.300-1.500	0.9993	0.100	0.300	2.1	0.8	-
NDBA	0.500-1.500	0.9992	0.150	0.500	2.2	3.3	-
Losartan tablets							
NDMA	0.500-1.500	0.9994	0.200	0.500	4.6	4.8	-
NDEA	0.500-1.500	0.9992	0.200	0.500	1.3	1.0	-
NEIPA	0.300-1.500	0.9993	0.100	0.300	1.4	0.9	-
NDIPA	0.300-1.500	0.9994	0.100	0.300	1.1	0.7	-
NDBA	0.500-1.500	0.9995	0.150	0.500	1.8	2.6	-
Ranitidine injectable							
NDMA	0.500-2.000	0.9998	0.200	0.500	0.8	0.8	-
NDEA	0.600-2.000	0.9994	0.200	0.600	5.0	5.4	-
DICM – UPLC-MS/MS							
Valsartan Tablet							
NDMA	0.053-1.000	0.9998	0.016	0.053	5.0	7.5	12.5
NDEA	0.060-1.000	0.9983	0.018	0.060	5.5	7.9	11.1
NEIPA	0.037-1.000	0.9984	0.011	0.037	3.1	6.6	9.1
NDIPA	0.050-1.000	0.9993	0.015	0.050	0.5	5.1	6.7
NDBA	0.045-1.000	0.9998	0.013	0.045	4.6	7.7	11.5
Losartan Tablet							
NDMA	0.053-1.000	0.9998	0.016	0.053	3.8	6.2	12.5
NDEA	0.060-1.000	0.9983	0.018	0.060	4.9	7.3	11.1
NEIPA	0.037-1.000	0.9984	0.011	0.037	2.7	6.8	9.1
NDIPA	0.050-1.000	0.9993	0.015	0.050	2.1	5.2	6.7
NDBA	0.045-1.000	0.9998	0.013	0.045	3.9	6.1	11.5
Ranitidine injectable							
NDMA	0.036-1.000	0.9990	0.011	0.036	3.9	7.8	18.2
NDEA	0.050-1.000	0.9991	0.015	0.050	5.0	8.4	13.3

[a] Calculated from signal-to-noise = 3.
[b] Calculated from signal-to-noise = 10.
[c] From peak areas for five determination of sample spiked at levels of 0.1 ng/mL of each N-nitrosamines.
[d] Calculated from the LODs obtained for Direct sample dissolution – UPLC-MS/MS divided by LODs of DICM-UPLC-MS/MS.

Table 2
Relative recovery studies of analytes in medicinal products using DICM-LC-MS/MS.

Analyte	Amount spiked (ng/mL)	Valsartan			Losartan			Ranitidine			Standard solution		
		Amount found (ng/mL)	Recovery (%, $n = 5$)	RSD (%, $n = 5$)	Amount found (ng/mL)	Recovery (%, $n = 5$)	RSD (%, $n = 5$)	Amount found (ng/mL)	Recovery (%, $n = 5$)	RSD (%, $n = 5$)	Amount found (ng/mL)	Recovery (%, $n = 5$)	RSD (%, $n = 5$)
NDMA	0.1	0.096	96.2	5.0	0.101	101.1	3.8	0.100	99.9	3.9	0.100	99.9	3.1
	0.5	0.522	104.4	4.7	0.524	104.8	4.2	0.482	96.5	4.9	0.501	100.2	2.7
	1.0	1.030	103.0	5.2	1.022	102.2	4.1	1.030	103.0	5.4	1.003	100.3	3.2
NDEA	0.1	0.104	104.1	5.5	0.100	99.5	4.9	0.104	103.9	5.0	0.102	102.0	3.5
	0.5	0.488	97.7	5.8	0.486	97.2	4.6	0.523	104.5	5.2	0.509	101.8	3.1
	1.0	0.998	99.8	4.7	0.964	96.4	5.1	0.977	97.7	5.3	0.998	99.8	2.9
NEIPA	0.1	0.105	105.2	3.1	0.103	102.6	2.7	-	-	-	0.099	99.1	3.6
	0.5	0.510	102.0	3.6	0.521	104.2	3.2	-	-	-	0.502	100.4	4.0
	1.0	1.030	103.0	3.2	1.044	104.4	3.5	-	-	-	1.017	101.7	3.9
NDIPA	0.1	0.097	97.3	0.5	0.099	99.3	2.1	-	-	-	0.100	99.9	2.5
	0.5	0.505	100.9	1.2	0.528	105.6	2.5	-	-	-	0.511	102.2	3.4
	1.0	1.049	104.9	1.1	1.041	104.1	2.2	-	-	-	1.001	100.1	3.3
NDBA	0.1	0.100	100.3	4.6	0.097	97.3	3.9	-	-	-	0.101	101.3	4.2
	0.5	0.520	104.0	3.9	0.495	99.1	4.3	-	-	-	0.512	102.4	3.5
	1.0	1.015	101.5	4.1	0.963	96.3	3.7	-	-	-	1.009	100.9	3.7

Table 3
Quantitative results for N-nitrosamines in commercial medicinal products.

Drug Sample	NDMA (μg/g)		NDEA (μg/g)		NDIPA (μg/g)		NEIPA (μg/g)		NDBA(μg/g)	
	Direct-UPLC-MS/MS	DICM-UPLC-MS/MS	Direct-UPLC-MS/MS	DICM-UPLC-MS/MS	Direct-UPLC-MS/MS	DICM-UPLC-MS/MS	Direct-UPLC-MS/MS	DICM-UPLC-MS/MS	Direct-UPLC-MS/MS	DICM-UPLC-MS/MS
Valsartan tablet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Losartan tablet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ranitidine Injectable	0.082 ± 0.004	0.083 ± 0.003	ND	ND	NS	NS	NS	NS	NS	NS

-ND: Not detected or below detection limit.
-NS: Not studied.
-Errors are standard deviations ($n = 3$).

ther using direct sample dissolution-UPLC-MS/MS or DICM-UPLC-MS/MS. All drug samples were measured in triplicates. The results are given in Table 3. The overall results obtained using DICM-UPLC-MS/MS are comparable to the results obtained employing the conventional direct-UPLC-MS/MS technique.

4. Conclusion

The present study demonstrates a new variation of dispersive liquid phase sample pretreatment technique termed as DICM for sample cleanup and preconcentration of N-nitrosamines in medicinal products. The developed approach has shown advantages in reducing extraction time and organic solvent consumption when compared to conventional SPE approach. In addition, improved detection and quantification limits were successfully achieved using proposed approached coupled with LC-MS/MS in comparison to previously reported techniques, as shown in Table S3. The incorporation of an inclusion complex formed between β-CD and targeted analytes during extraction successfully overcomes the limitations of conventional DLLME technique, whereby the overall extraction efficiency has been improved especially when dealing with less hydrophobic analytes of interest. The proposed new DICM-UPLC-MS/MS approach was successfully applied to monitor ARBs sample tablet and ranitidine injectable with enhanced method LOQ when compared to the validated direct sample dissolution-UPLC-MS/MS technique recommended in the USP General Chapter 1469. The method is deemed suitable for routine drug monitoring, which meets both the current and more stringent regulatory limit values enforced by authorities worldwide.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Karen Sze Jie Tay: Conceptualization, Writing – original draft. **Michael C. Breadmore:** Writing – review & editing. **Ee Shan Soh:** Writing – review & editing. **Hong Heng See:** Conceptualization, Writing – review & editing.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463605.

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