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ISSN 1506–9680
VIA MEDICA

New synthesis route of active substance d,l-HMPAO for preparation Technetium Tc99m Exametazime

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[Received 28 XII 2016; Accepted 25 IV 2017]

Abstract

BACKGROUND: Technetium Tc99m Exametazime (^{99m}Tc-HMPAO) is currently used as a radiopharmaceutical for determining regional cerebral blood flow and for the labelling of autologous leucocytes for infection and inflammation imaging. The HMPAO ligand exists in two diastereomeric forms: d,l and meso. Usually, the substance is obtained in low chemical yield in a time consuming procedure. Furthermore, the final product still contains some amounts of the meso-form. The aim of this study was to develop the efficient, reliable and fast method for isolation of the d,l-HMPAO, which would provide the ligand with high purity and free from the meso-diastereomer.

MATERIAL AND METHODS: The mixture of the meso- and d,l-HMPAO was synthesized in two-steps by condensation of propanediamine with keto-oxime and the reduction of the obtained bisimine. The d- and l-enantiomers were separated individually directly from this mixture by repeated crystallizations from ethanol as their tartrate salts and pooled together in equal proportions. That substance was characterized for its identity and isomeric purity using IR, HPLC and GC methods. The meso-free d,l-HMPAO was used for the preparation of the radiopharmaceutical freeze-dried kit for technetium-99m radiolabelling. Quality assessment of obtained ^{99m}Tc-d,l-HMPAO complex was performed according to the current Ph.Eur. monograph 1925 and USP monograph — Technetium Tc99m Exametazime Injection. To verify its biological activity, the kit-prepared ^{99m}Tc-d,l-HMPAO has been used for the white blood cell (WBC) labelling.

RESULTS: According to the proposed synthesis route the d,l-HMPAO was obtained with around 18–20% yield in the total time of 10 days. The ligand identity was confirmed and the HPLC analysis revealed more than 99% chemical purity. The undesired meso-form was not detected. Freeze dried kit formulation for ^{99m}Tc-labelling of d,l-HMPAO has been established and four batches of kits were manufactured. The radiochemical purity of ^{99m}Tc-d,l-HMPAO complex was high (> 95% of lipophilic technetium-99m exametazime). Brain uptake in rats reached 2.1 ± 0.3%. The *in vitro* labelling of WBC resulted in 68.3 ± 6.6% yield.

CONCLUSION: A new synthesis method of d,l-HMPAO, drug substance for technetium-99m exametazime preparation has been developed.

KEY words: ^{99m}Tc-d,l-HMPAO, chemical synthesis, ^{99m}Tc-exametazime, brain SPECT, infection imaging

Nucl Med Rev 2017; 20, 2: 88–94

Background

Technetium-99m exametazime (^{99m}Tc-HMPAO) has been introduced as a tracer for brain imaging studies in humans as early as 1985 [1, 2] and its diagnostic utility has been very well documented [3, 4]. Currently, it is recommended by the European Association of Nuclear Medicine (EANM) for brain perfusion studies using Single Photon Emission Computed Tomography (SPECT) [5]. It is used to diagnose abnormalities in the regional cerebral blood flow such as those occurring after a stroke and other cerebrovascular diseases including epilepsy, Alzheimers disease and other forms of dementia, transient ischemic attacks, migraine and brain tumors. Another application is the labelling of autologous leucocytes for infection and inflammation imaging [6].

The synthesis of the active substance, ligand HMPAO (hexamethylpropylene amineoxime) has been first described by Novotnik

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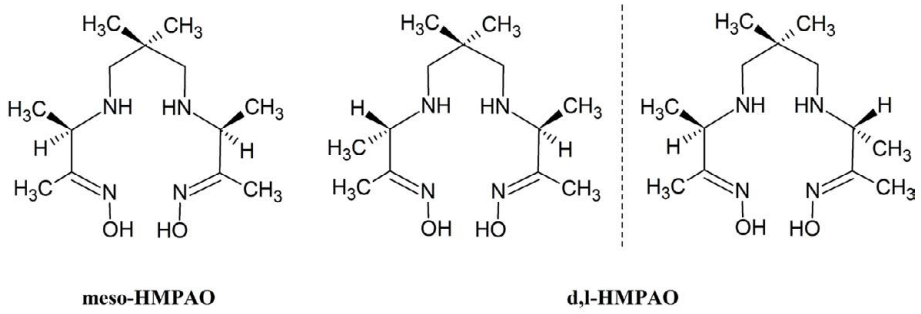

Figure 1. Diastereomers of HMPAO [3]

et al. [1, 7]. As elucidated by Neirinckx et al. [3], HMPAO has two chiral centers producing the meso- (R,S), d- (R,R) and l- (S,S) diastereomers (Fig. 1).

It was originally confirmed that technetium-99m complexes of these two HMPAO diastereomers (meso- and d,l racemate) showed different properties in vivo both in rats [3, 4] and in humans [8]. They rapidly and easily diffused across the blood-brain barrier (BBB) at normal flow rates and had similar brain uptake. However, the d,l-complex revealed superior brain uptake and retention (4.1% after 8h post injection) compared to the complexes generated from meso-isomer (1.7%) and stereoisomeric mixtures (1.9%) [8]. Thus, the meso-diastereomer component decreased the radiopharmaceutical concentration in the brain. In addition, the later studies indicated that brain uptake of ^{99m}Tc-complexes formed from separated d- and l-enantiomers differed. The cerebral uptake of ^{99m}Tc-l-HMPAO and ^{99m}Tc-d,l-HMPAO complexes was similar but notably it was higher (1.5 fold) than for the isolated ^{99m}Tc-d-HMPAO complex [9, 10]. For these reasons, to reach the optimum brain uptake, the HMPAO active substance should preferably be composed of d- and l-enantiomers without contribution of the meso-form. Consequently, the ^{99m}Tc-meso-HMPAO is specified as radiochemical impurity in the Technetium Tc99m Exametazime Injection monograph, and its content should not be more than 5% in the final preparation used for investigations in humans [11, 12]. Hence, purity of HMPAO used as active substance for manufacturing of dry kits for radiopharmaceutical preparation is a critical parameter.

The previously reported procedures of d,l-HMPAO purification from the meso-form involved the repeated fractional crystallizations of crude HMPAO from organic solvents [7, 13, 14]. This method is very time consuming and affords many fractions of varying crystallinity and diastereomeric composition. Hence, it results in the drastic reduction of the synthesis yield (overall yield reported was around 1.2 %) and the final d,l-HMPAO fraction still contains small (3–5%) amounts of the meso-form [13, 14].

The aim of this study was to develop the efficient, reliable and fast method for isolation of d,l-HMPAO, which would provide product with the high purity and which would be free from undesired meso-diastereomer. Herein, we report the new purification procedure. The protocol was based on the individual separation of d- and l-enantiomers by crystallization of their tartrate salts directly from the meso,d,l-mixture. The method has been optimized against the in-house prepared standard substances (Chemical Reference Standards, CRS of each d,l-, meso-, d- and l-isomers). The purified d- and l-isomers were than pooled together to form the active pharmaceutical ingredient suitable for pharmaceutical kit development. The quality of such obtained active substance synthesized in our laboratory was then confirmed *in vitro* by ^{99m}Tc labelling of leucocytes and *in vivo* by the biodistribution studies using the kit prepared complex of ^{99m}Tc-d,l-HMPAO.

Material and methods

Chemicals and analytical methods

All reagents and solvents used in reactions were of commercial quality and purchased from Sigma-Aldrich Co. (USA) and POCH (Poland). ^{1}H NMR spectra were recorded using a Bruker 500 MHz spectrometer. CH_3OD was used as solvent, and chemical shifts (δ) were reported in ppm relative to TMS as an internal standard. IR spectra were recorded using Nicole iS10-Thermo Scientific spectrometer. Mass spectra were obtained on API 365PESciex turboionspray tandem mass spectrometer (ESI method). Melting point was determined using OptiMelt (SRS) apparatus. Analytical HPLC were run in a system comprising Shimadzu LC-20AD pump, SPD-M20A diode array detector and CBM-20A controller. Content of residual solvents was measured using GC system (GC 2010, Shimadzu) comprising a flame ionization detector (FID), a split/splitless injection unit and a headspace HS40 (Perkin Elmer).

Synthesis of d,l-HMPAO racemate

The d,l-HMPAO was synthesized according to published procedures [3, 7, 14] with minor modifications. The synthetic route is shown in Figure 2. Briefly, this two-step synthesis involved condensation of propanediamine (compound 2) with two molecular equivalents of the keto-oxime (compound 1) in the first step, followed by the reduction of the obtained bisimine (compound 3) with sodium borohydride. These reactions yielded HMPAO (compound 4) as a mixture of meso- and d,l-diastereomers.

In the next steps the stereoisomers were separated using chemical methods as presented in Figure 3. The d- and l-enantiomers were separated individually directly from the mixture of meso-, d- and l-HMPAO by repeated crystallizations from ethanol as their (–) tartrate and (+) tartrate salts respectively [7, 15]. The tartrate salts were converted into the free base form by treating the aqueous solutions of the ligand with excess of solid sodium carbonate followed by the extraction and crystallization from acetonitrile.

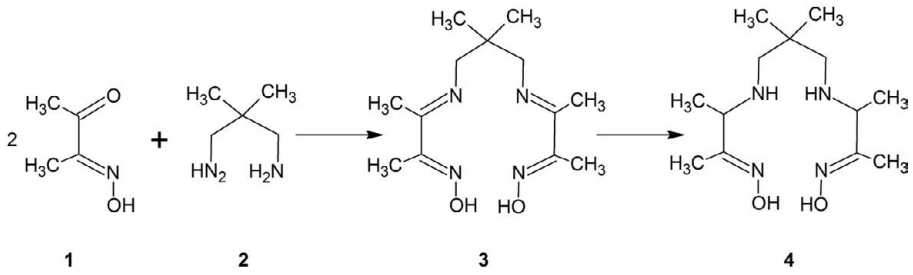

Figure 2. Synthesis route of HMPAO [3]

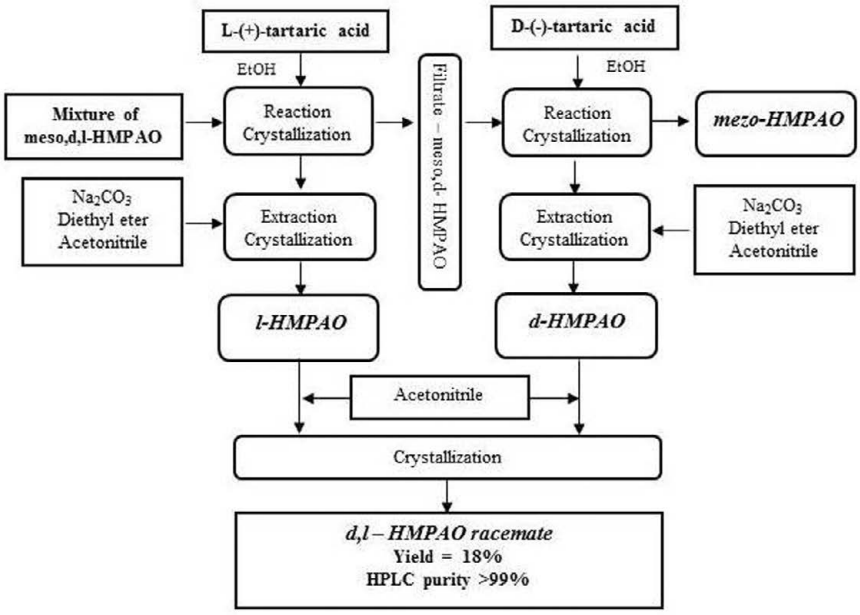

Figure 3. Flow-chart of the d,l-HMPAO purification process

Characterization of d,l-HMPAO as active substance

The quality testing methods of d,l-HMPAO active substance were standardized against Chemical Reference Standard of each d,l-, meso-, d- and l-isomers. The in-house prepared CRSs were characterized in detail in accordance with the relevant guidelines of European Pharmacopoeia [16]. Table 1 presents the summary of data for each CRS prepared. The identity of these compounds was confirmed using the following methods: IR, ^{1}HNMR, melting point and optical rotation.

The identity of d,l-HMPAO active substance was confirmed by the IR spectroscopy, melting point measurement and comparison of the retention time with the retention time of d,l-HMPAO CRS. The purity was determined by the analytical HPLC method (assay and organic impurities) and by the GC method (residual solvents). The stereochemical purity of the separated diastereomers was determined using the analytical chiral HPLC column (Chiralpak AD, Daicel Chemical Industries LTD) and hexane/ethanol/n-butylamine mixture (85/15/0.05%) as the mobile phase. The flow rate of 1.2 mL/min and UV detection at 220 nm were used. For analysis of residual solvents (diethyl ether, acetonitrile and ethanol) the DB-624 column (Agilent) was used.

Freeze dried kit development and radiolabelling

The meso-free d,l-HMPAO was used as an active substance for the preparation of the radiopharmaceutical freeze-dried kit for technetium-99m radiolabelling. The amount of active substance in the single kit vial was 0.5 mg, similar to the commercially available formulation [17]. The influence of stannous chloride dihydrate amount on the kit performance was investigated in the range from 0.003 mg to 0.007 mg. Freeze-drying was carried out in Christ Alpha 1–4 LSC freeze dryer. The assay of tin(II) as $SnCl_2 \times 2H_2O$ in the final kit formulation was determined using ICP-OES OPTIMA 7300 DV spectrometer. Water content in the kits after lyophilization was determined by Karl Fischer method using coulometric titration with thermal extraction (852 Titrando, Methrom AG). For radiolabelling the content of the freeze-dried kit was dissolved in around 4.5 mL of 0.9% NaCl immediately followed by addition of around 0.5 mL of eluate of sodium pertechnetate, $^{99m}TcO_4^-$ with the radioactivity

Table 1. Characteristics of Chemical References Substances (CRS) d-HMPAO, l-HMPAO, d,l-HMPAO and meso-HMPAO.

Characteristic	Compound			
	d-HMPAO	l-HMPAO	meso-HMPAO	d,l-HMPAO
Purity (HPLC) (%)	≥ 99.0	≥ 99.0	≥ 91.5	≥ 99.0
Content of meso-HMPAO (HPLC) (%)	≤ 1.0	≤ 1.0	-	≤ 1.0
Assay (HPLC, GC and elemental analysis) (%)	≥ 97.0	≥ 97.0	≥ 90.0	≥ 97.0

of 740-925 MBq (20–25 mCi). Then the content was gently swirled at ambient temperature for 5 min.

Quality assessment of the ^{99m}Tc-d,l-HMPAO complex

The tests for identity, radiochemical purity and pH of ^{99m}Tc-d,l-HMPAO were performed according to Ph.Eur. monograph 1925 [11].

Biodistribution of ^{99m}Tc-d,l-HMPAO

Biodistribution was evaluated in Wistar rats (male, weight 130–190 g) according to USP monograph [12]. The animal experiments were approved by The 4th Local Animal Ethics Committee in Warsaw (authorization number 78/2011) and carried out in accordance with the principles of good laboratory practice (GLP). In a single experiment 3 rats were injected (tail vein) with around 18.5–30 MBq of ^{99m}Tc-d,l-HMPAO in 100 μL, each. Animals were euthanized by overdose of isoflurane at five to ten minutes after the injection (p.i.). Samples of brain, liver and intestines (including gall bladder) were collected, weighed and their radioactivity was measured in the NaI gamma counter supplied with adapter for the whole body measurement. The organ uptake values were expressed as the percent of injected dose per organ (%ID).

White blood cell (WBC) labelling with ^{99m}Tc-d,l-HMPAO

The radiolabelling of WBC was performed according to the EANM guidelines for labelling of leucocytes with ^{99m}Tc-d,l-HMPAO [6]. The samples of fresh blood were collected from healthy volunteers. A 60-mL syringe was filled with 9 mL of acid citrate-dextrose anticoagulant (ACD, Sigma-Aldrich Co., USA) solution and 51 mL of the volunteers blood. Then the syringe with the blood–ACD solution was gently rotated. Next, the 15 mL of the blood–ACD solution was transferred into a Falcon centrifugation tube and centrifuged at 2,000g at room temperature for 10 min. The cell-free plasma (CFP) was separated from the pellet and removed. Erythrocytes were allowed to sediment with the aid of 2-hydroxyethyl starch (10% HES, pharmaceutical grade, Sigma-Aldrich Co., USA) and leucocyte-rich plasma (LRP) from blood was collected. The obtained LRP was gently resuspended with PBS and centrifuged twice at 150 g for 10 min. After that the supernatant from the leucocyte pellet was removed and the pellet was gently resuspended in 1 mL of fresh PBS and used for labelling. A 1 mL of freshly prepared ^{99m}Tc-HMPAO (approximately 750–1.000 MBq) in saline solution was added to the mixed leucocyte cell suspension and incubated for 10 min at room temperature with gently swirling to prevent sedimentation of the cells. After the incubation was completed, at least 3 mL of PBS were added and centrifuged at 150 g for 5 min. The supernatant containing unbound ^{99m}Tc-HMPAO was removed, the radioactivity of the pellet and the supernatant was measured to calculate the LE. The experiment was performed in triplicate. The labelling efficiency (LE) was calculated as LE (%) = radioactivity in the pellet / (radioactivity in the pellet + radioactivity in the supernatant) × 100%.

Results

Using the synthetic route presented in this study, the intermediate bisimine product (compound 3) was obtained with 50% yield, and then reduced into a diastereomeric mixture (compound 4) with 37% yield. The individual d- and l-enantiomers were separated directly from the crude meso,d,l-HMPAO mixture (containing 40–50% meso-HMPAO) by techniques involving the use of optically pure D-(-)- and L-(+)-tartaric acid as resolving agent [7, 15]. The obtained d-HMPAO D-(-)-tartrate and l-HMPAO L-(+)-tartrate salts were purified by repeated crystallization and converted into free base to give pure d- and l-HMPAO, without contribution of meso-isomer. The final mixture of d,l-HMPAO was obtained with around 18–20% yield from equal portions of d- and l-enantiomers in the total time of 10 days. The identity of d,l-HMPAO was confirmed by melting point, IR, HPLC and GC analysis. The very sensitive and selective analytical HPLC method, demonstrated chemical purity of d,l-HMPAO of more than 99% (Figure 4). The content of undesired meso-form in the final drug substance was less than 0.1% (limit of detection). The residual solvents were detected at the level of 600 ppm for ethanol, of 100 ppm for acetonitrile and 10 ppm for diethyl ether, which were well below acceptance limits stated by Ph. Eur. of 5000 ppm for ethanol and diethyl ether and 410 ppm for acetonitrile.

The influence of stannous chloride dihydrate content on the quality of ^{99m}Tc-d,l-HMPAO was investigated in the series of batches of kits freeze-dried with various amounts of $SnCl_2 \times 2H_2O$ (0.003, 0.005 and 0.007 mg). The radiochemical purity and biodistribution results were dependent on the content of stannous chloride dihydrate in the kit formulation. In particular, the increase of $SnCl_2 \times 2H_2O$ from 0.003 to 0.007 mg in the kit resulted in decreasing radiochemical purity and in lower brain uptake, as presented in Table 2.

The optimum chemical composition of the kit appeared to be as follows: 0.5 mg of d,l-HMPAO; 4.5 mg of sodium chloride, 0.003 mg of stannous chloride dihydrate, freeze-dried and sealed under nitrogen as an inert atmosphere.

Four batches of kits were manufactured according to that formulation and tested for their quality. The summary of results is presented in Table 3, each value is the average result of tests performed independently in 4 manufactured batches (standard deviations reflect the inter-batch variability). The impurities denoted as B, D and E

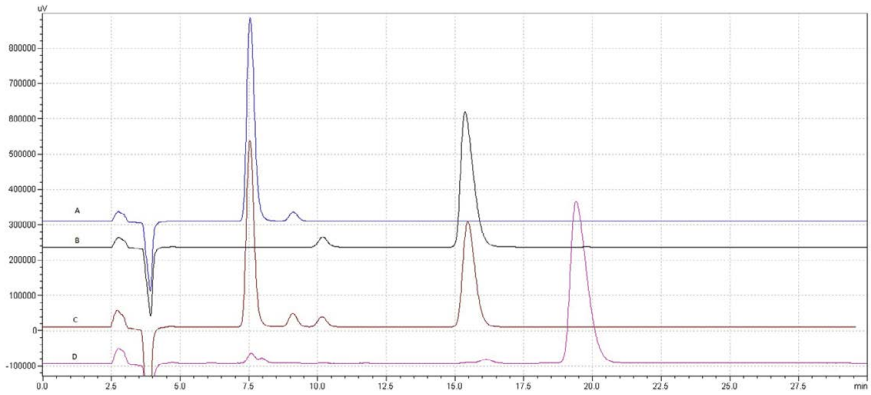

Figure 4. Comparative chromatograms of HMPAO diastereomers: (A) l-HMPAO, (B) d-HMPAO, (C) d,l-HMPAO, (D) meso-HMPAO

Table 2. The influence of $SnCl_2$ x $2H_2O$ content on the performance of frieze-dried kits (n = 3)

Test	Requirements	Amount of $SnCl_2$ x $2H_2O$		
		0.003 mg	0.005 mg	0.007 mg
Radiochemical purity:	Ph. Eur. 1925			
— [^{99m}Tc] pertechnetate ion (C)	≤ 10%	5.1 ± 3.7%	2.2 ± 0.1%	0.7 ± 0.3%
— impurities B+D+E	≤ 10%	8.4 ± 1.9%	19.3 ± 1.2%	67.9 ± 0.8%
— isomer meso of lipophilic technetium-99m exametazime (A)	≤ 5%	0.0 ± 0%	0.0 ± 0%	0.0 ± 0%
— lipophilic technetium-99m exametazime and its isomer meso	≥ 80%	86.5 ± 4.2%	78.5 ± 4.9%	31.4 ± 0.6%
Biological distribution:	USP			
— brain	≥ 1.5% D	1.9 ± 0.0%	1.4 ± 0.3%	1.0 ± 0.2%
— liver	≤ 15% ID	9.0 ± 1.9%	9.6 ± 0.3%	34.6 ± 1,4%
— intestine	≤ 20% ID	14.6 ± 3.5%	12.4 ± 1.1%	8.8 ± 0.5%
Water content	≤ 1.0%	0.6 ± 0.1%	2.0 ± 0.1%	2.4 ± 0.2%

Table 3. Quality control results of frieze-dried kits (n = 4)

Test	Requirements	Results
Radiochemical purity:	Ph. Eur. 1925	
— [^{99m}Tc] pertechnetate ion (C)	≤ 10%	1.0 ± 0.7%
— impurities B+D+E	≤ 10%	3.8 ± 1.9%
— isomer meso of lipophilic technetium-99m exametazime (A)	≤ 5%	0.0 ± 0
— lipophilic technetium-99m exametazime and its isomer meso	≥ 80%	95.2 ± 1.8%
Biological distribution:	USP	
— brain	≥ 1.5% ID	2.1 ± 0.3%
— liver	≤ 15% ID	8.5 ± 0.6%
— intestine	≤ 20% ID	12.7 ± 1.7%
Water content	≤ 1.0%	0.7 ± 0.1%

are: ^{99m}Tc in colloidal form, non-lipophilic ^{99m}Tc-HMPAO complex and meso-isomer of non-lipophilic ^{99m}Tc-HMPAO complex, respectively, in accordance with the Ph.Eur. monograph 1925.

The identity of ^{99m}Tc-d,l-HMPAO complex was confirmed by HPLC test (Figure 5). The radiochemical purity of ^{99m}Tc-d,l-HMPAO complexes obtained from the prepared kits was high (> 95% of lipophilic technetium-99m exametazime). The impurity A (isomer meso of lipophilic technetium-99m exametazime) was not detected and the contents of the other impurities (B + D + E) were well below the acceptance limit of 10%.

In all 4 tested batches the high brain uptake (about 2%) at 15 min p.i. was observed. Liver and intestine uptake was about 8 and 13%, respectively. The WBC labelling with the kit obtained ^{99m}Tc-d,l-HMPAO resulted in 68.3 ± 6.6% (n = 3) yield.

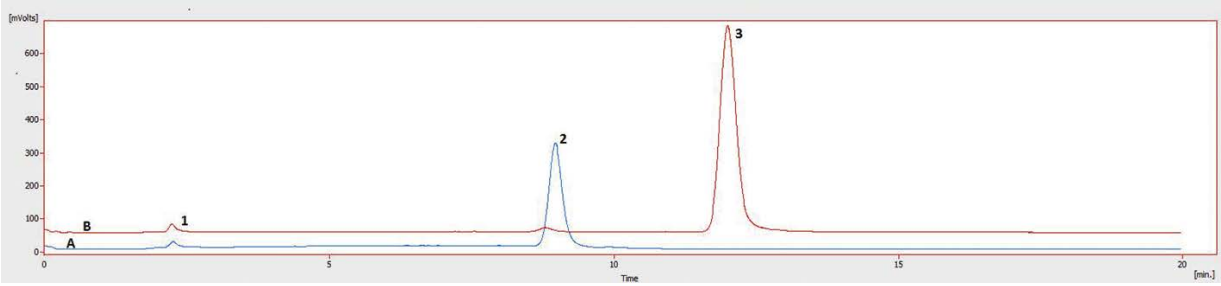

Figure 5. Comparative chromatograms of (A) ^{99m}Tc-d,l-HMPAO obtained from the kit (blue line) and (B) the standard of ^{99m}Tc-meso-rich HMPAO (red line). Peaks: 1 — [^{99m}Tc]-pertechnetate ion, 2 — ^{99m}Tc-d,l-HMPAO, 3 — ^{99m}Tc-mezo-HMPAO

Discussion

Using a novel purification method, the active substance of d,l-HMPAO was prepared with high yield of around 18–20% in the total time of 10 days and with purity higher than 99%. This substance was also free from the meso diastereomer. The procedures described in literature so far enable the separation of d,l- and meso-HMPAO. However, due to the formation of amorphous phase during the crystallization from organic solvents the overall yield of the desired diastereomer is drastically reduced, giving an overall yield of about 1.2% [13, 14]. Bearing that in mind, the method proposed in this report appears superior with the one order of magnitude higher yield. The "classical" method is also very time consuming (total purification time can be up to one month) and it is difficult to reproduce and validate. Moreover, the d,l-fractions still contains significant amounts (about 3–5%) of the meso-form [13], which is not the case in our development. The advantage of the method described herein is a more reliable separation of pure d,l-HMPAO. Using the optically active tartaric acids we have achieved very selective and simple resolving of pure d- and l-enantiomers. Furthermore, the repeated crystallization of the individual tartrate salts from ethanol led to the practically complete removal of meso-isomer.

The value of the new synthesis route, leading to the active substance for ^{99m}Tc-d,l-HMPAO, has been clearly demonstrated in *in vitro* and *in vivo* studies. It is well known that the primary lipophilic complex of ^{99m}Tc-d,l-HMPAO is very rapidly converted to the secondary hydrophilic complex in the presence of plasma [3]. Factors which have an influence on the decomposition of the lipophilic complex are as follows: ionizing radiation, quantity of the Sn (II) ions and pH. Similarly to the commercially available kits, the ^{99m}Tc-d,l-HMPAO complex obtained from our kit formulation was stable up to 30 minutes after preparation [17]. We have also confirmed that the stability over time of ^{99m}Tc-exametazime preparation may be significantly extended by the addition of a freshly prepared stabilizing mixture of methylene blue solution [18] (the results are not shown).

According to USP monograph, the required uptake of ^{99m}Tc-d,l-HMPAO in the brain should not be less than 1.5% ID, not more than 20% ID in the intestines, and not more than 15% ID in the liver [12]. In our study the primary complex of ^{99m}Tc-d,l-HMPAO exhibited 2.1%ID brain uptake at 10 min p.i., which is well over the required limit, both the liver and intestine uptake were also within the required limits. When the kit obtained ^{99m}Tc-d,l-HMPAO was used for the radiolabelling of WBC, the labelling yields of 68.3 ± 6.6% (n = 3) were reached, which is in accordance with the recommendations where radiolabelling efficiency between 40% and 80% is expected [6]. Despite the promising *in vitro* and *in vivo* results the diagnostic utility of ^{99m}Tc-d,l-HMPAO containing the active substance synthetized by a modified purification route still needs to be confirmed in the clinical evaluation of brain perfusion abnormalities.

Conclusion

In this study, the new and efficient procedure for preparation of HMPAO ligand (hexamethylpropylene amineoxime) rich in d- and l-enantiomers and free from the meso-form has been reported. The freeze-dried kit formulated from such obtained ligand used as an active substance resulted in the reproducible preparation of drug product Technetium Tc99m Exametazime Injection of high radiochemical purity, favorable brain uptake in rodents and the high *in vitro* labelling yield of WBC.

References

1. Nowotnik DP, Canning LR, Cumming SA, et al. Development of a 99Tcm-labelled radiopharmaceutical for cerebral blood flow imaging. Nucl Med Commun. 1985; 6(9): 499–506, indexed in Pubmed: 3877892.
2. Holmes RA, Chaplin SB, Royston KG, et al. Cerebral uptake and retention of 99Tcm-hexamethylpropyleneamine oxime (99Tcm-HM-PAO). Nucl Med Commun. 1985; 6(8): 443–447, indexed in Pubmed: 3877890.
3. Neirinckx RD, Canning LR, Piper IM, et al. Technetium-99m d,l-HM-PAO: a new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. J Nucl Med. 1987; 28(2): 191–202, indexed in Pubmed: 3492596.
4. Neirinckx RD, Harrison RC, Forster AM, et al. et al.. A model for the in vivo behavior of Tc-99m d, l-HMPAO on man. J Nucl Med. 1987; 28: 559.
5. Kapucu OL, Nobili F, Varrone A, et al. EANM procedure guideline for brain perfusion SPECT using 99mTc-labelled radiopharmaceuticals, version 2. Eur J Nucl Med Mol Imaging. 2009; 36(12): 2093–2102, doi: 10.1007/s00259-009-1266-y, indexed in Pubmed: 19838703.
6. de Vries EFJ, Roca M, Jamar F, et al. Guidelines for the labelling of leucocytes with (99m)Tc-HMPAO. Inflammation/Infection Taskgroup of the European Association of Nuclear Medicine. Eur J Nucl Med Mol Imaging. 2010; 37(4): 842–848, doi: 10.1007/s00259-010-1394-4, indexed in Pubmed: 20198473.
7. Canning LR, Nowotnik DP, Neirinckx RD, et al. Complexes of technetium-99m with propylene amine oximes. European Patent. ; 0194843.

8. Sharp PF, Smith FW, Gemmell HG, et al. Technetium-99m HM-PAO stereoisomers as potential agents for imaging regional cerebral blood flow: human volunteer studies. J Nucl Med. 1986; 27(2): 171–177, indexed in Pubmed: 3712035.
9. Hoffman TJ, Corlija M, Reitz R, et al. Biological evaluation of the Tc-99m complexes of the D and L isomers of HMPAO. J Nucl Med. 1992; 33: 1032.
10. Kang CS, Chang YS, Jeong JM, et al. Synthesis of d- and l-form of 99mTc-HMPAO, and comparison of brain uptake. Korean J Nucl Med. 2001; 35(1): 69–74.
11. European Pharmacopoeia, monograph 1925 Technetium (99mTc) exametazime injection.
12. The U.S. Pharmacopeia (USP) monograph: Technetium Tc 99m Exametazime Injection.
13. Banerjee S, Samuel G, Kothari K, et al. On the synthesis, isolation, and radiochemical studies for the preparation of in-house kits for 99mTc-meso- and d,l-HMPAO: a few additional observations. Nucl Med Biol. 1999; 26(3): 327–338, indexed in Pubmed: 10363805.
14. IAEA Technical Reports Series no. 466. Technetium-99m Radiopharmaceuticals: Manufacturing of Kits. , Vienna 2008: 140–143.
15. Nowotnik DP, Nanjappan P, Zeng W, et al. Separation of the Stereoisomers of Hexamethyl-Propyleneamine Oxime (HM-PAO) by High-Performance Liquid Chromatography. Journal of Liquid Chromatography. 1995; 18(4): 673–687, doi: 10.1080/10826079508009264.
16. European Pharmacopeia, monograph 5.12. Reference Standards.
17. SmPC GE Healthcare, Ceretec. www3.gehealthcare.com.
18. Seguí GV, Engroñat RM, Muñoz AF, et al. [Stabilization of 99m Tc-HMPAO with methylene blue: its use in leukocyte labelling)]. Rev Esp Med Nucl. 1998; 17(4): 261–264, indexed in Pubmed: 9721341.