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Analytical Methods for Determination of Entacapone in Pharmaceuticals and Urine

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Abstract

Capillary electrophoresis (CE), high performance thin layer chromatography (HPTLC) and spectrophotometric (SPM) methods were compared for the determination of entacapone in pharmaceutical preparations. And ultrasonication assisted extraction (UAE) coupled with CE determination of cis and trans isomers of entacapone in urine sample was optimized. Resolution of entacapone isomers could not be achieve on RP-HPTLC developed with a native or modified cyclodextrins as a mobile phase additive. Different types aqueous and non-aqueous background electrolytes were tested for the resolution of isomers by CE. Good resolution (Rs ~4.9) was achieved on using a non-aqueous background electrolyte (NBGE). Tailing and concentration dependent migration were observed on silica gel HPTLC plates, due to strong adsorption of entacapone. However, the plate developed with tetrahydrofuran (THF), a good baseline separation was achieved. Good recovery of entacapone was achieved in tablet dosage by using non-aqueous extraction solvent system (NAES). All the methods (SPM, HPTLC and CE) the correlation coefficient (R2) were > 0.9993. SPM method is found be simple, convenient and faster than HPTLC and CE for the determination of entacapone in tablet dosage forms. However, the CE method alone found to be useful for separation and determination of cis and trans isomer in tablets and urine samples and it is simple, fast, cost-effective and efficient.

Keywords:

Entacapone isomers; Capillary electrophoresis; High performance thin layer chromatographic; Spectrophotometric; Ultrasonication assisted extraction

1. Introduction

Entacapone, [2-cyano-3-(3,4-dihyroxy-5-nitrophenyl)-N,N-diethyl-2-propenamide] is a potent inhibitor of catechol-O-methyltransferase (COMT) [1]. Trans isomer of entacapone is used as an adjunct in therapy of Parkinson's disease. It available both in single entity as well as combination with levodopa and carbidopa in tablet dosage forms. Even though, both isomers are pharmacologically active, the trans isomer alone is used as an adjunct drug and the cis isomer controlled to be less than 0.5% in pharmaceutical dosage forms. The trans isomer prevents levodopa metabolize to 3-o-methyldopa, a toxic metabolite. Orally

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administrated trans isomer is almost completely metabolized and available around 0.2% in unchanged form in urine. Therefore, a simple and efficient extraction technique is required for its determination**..** The chemical structure of *trans* and *cis* entacapone is shown Fig.1

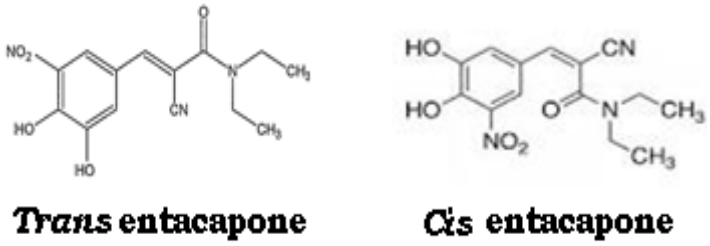

Fig.1. Chemical structure of *trans* and *cis* entacapone

A few HPLC, SPM and electrochemical methods have been reported for the determination entacapone in pharmaceutical dosage forms [2-6]. CE [7, 8], HPLC [9] and LC-MS [10] methods were reported for the determination of entacapone isomers in urine and plasma. Although the LC-MS or CE-MS are more sensitive than LC or CE and their availability in medium or small-scale pharmaceutical labs are very rare.

The application of CE in pharmaceutical analyzes [11-14] are increasing year-by-year due to its high resolving power, wide application, low running cost and use of eco-friendly solvent system for separation. It requires a low volume of buffer and less quantity markers or standards for the analysis. Further optimization of separation conditions is easy and faster than other separation techniques. The application CE for the determination cis and trans isomer in tablets dosage forms is not available in literature.

Direct determination of entacapone glucuronide metabolites in urine sample considered difficult to due to non-availability of glucuronide standards commercially. Therefore, the standards were either synthesized [15] or enzymatic hydrolysis [8] for determination and identification. Prior to analysis of CE-MS or LC-MS, the analytes were extracted from urine sample by using solid phase extraction (SPE) and solvent extraction methods. The extraction procedures described therein were tedious and time consuming. Therefore, a simple and efficient extraction technique is required for the extraction of entacapone isomers in urine samples.

Recent years, UAE has received a considerable attention due to its potential application for extraction of trace amount of compounds in aqueous samples [16-19]. The method involves simple steps: adjustment of pH, addition of salt and water immiscible organic solvent and ultrasonication. The advantage of the method is its simplicity, cost-effectiveness and efficient extraction of hydrophobic compounds.

Entacapone is sparingly soluble in acetone, slightly soluble in anhydrous ethanol and insoluble in water and not stable in phosphate buffer (pH 7.4) [5]. Therefore, a suitable extraction medium is necessary to extract entacapone in tablet dosge form as well as in urine samples.

The aim of present study was evaluation of CE and HPTLC methods for resolution and determination of cis and trans isomers of entacapone in tablet dosage forms and urine. Comparison of SPM, HPTLC and CE methods for the determination of entacapone in tablet dosage forms.

2. Materials and methods

2.1. Chemicals

All the chemicals and solvents were of analytical or chromatographic grade and used as it is without any purification. Sodium tetraborate (STB) was purchased from Sigma-Aldrich, Bangalore, India. Methanol, 1-propanol, sodium hydroxide, chloroform and hydrochloric acid were procured from SD Fine Chemicals, Mumbai, India. Hydroxyl propyl-β-cyclodextrin, heptakis(2,6-di-o-methyl)-β-cyclodextrin and α-cyclodextrins were from Sigma-Aldrich. Enatacapone tablets were obtained from local medical stores. Entacapone isomers were gifted from Sipra laboratories, Hyderabad.

2.2. Sample preparation

2.2.1. Standard and BGE solution

A stock standard solution of entacapone isomer of each (0.5 mg mL^{-1}) was prepared in methanol. Required concentrations of working standards of individual and mixtures were prepared with the same solvent before the analysis.

Non-aqueous background electrolyte (NBGE) : 14.0 mM STB dissolved in 4 mL of 0.1 M NaOH followed by 30 mL of methanol, 20 mL of 1-propanol were added and ultrasonicated for 5 min. Prior to use, the NBGE was purged with helium for 2 min to remove dissolved gases and filtered. The apparent pH* of the solution was 10.5. All the solutions were stored in an airtight amber colored bottle inside a refrigerator.

2.2.2 Entacapone tablets

Twenty tablets were weighed accurately and ground to fine powder. A portion of the powder that equivalent to about 5.0 mg of entacapone was transferred to 15 mL centrifuge tube and make up to the mark 10 mL with non-aqueous extraction solvent sytem (NAES) consisting a mixture of 7.4% 0.1 NaOH, 55.6% methanol and 37.0% 1-propanol, i.e NBGE system without STB. Sample was vertex-mixed for 2 min and ultrasoniction, alternatively for 5 min. The procedure was repeated for three times and subsequently centrifuged for 5 min. The required concentrations of working standard solutions were prepared in NAES for SPM analysis and in methanol for CE and HPTLC analysis.

2.2.3. Urine

Blank urine samples were obtained from volunteers and recovery studies were performed by spiking a known amount in the range of 5.0 to 50.0 µg mL^{-1} entacapone isomers.

A volume (8.0 mL) of spiked urine sample was transferred to a centrifuge tube and adjusts the pH to 3.5 with 0.1 N HCl and 300.0 mg sodium chloride was added. After vortex-mixed, 1.5 ml chloroform was added. Samples were again vertex-mixed 30 s and ultrasonication, alternatively for 5 min. The procedure was repeated for three times and subsequently centrifuged for 5.0 min. One milliliter of chloroform layer was carefully removed with help of a syringe and evaporated the solvent. Prior to analysis, the sample was dissolved with a known volume of methanol and filtered. To prevent isomerization, the samples were protected from light during extraction.

2.3. HPTLC analysis

Camag (Muttenz, Switzerland) HPTLC system equipped with Linomat IV system (sample applicator) and Camag TLC scanner III was used for TLC analysis. Pre-coated HPTLC silica gel F254, aluminium plates were used for separation. Tetrahydrofuran, a single solvent system was used for the development. Detection wavelength was set at 300 nm and winCATS software (v 1.4.4 Camag) was used for operation and collecting analytical data.

2.4. CE analysis

Prince Technologies (The Netherlands) CE system equipped with an auto sampler and photodiode array detector was used for analysis. Capillary tube compartment and sample tray and temperature were set at 20°C and 25°C, respectively. An uncoated fused-silica capillary (75 μM I.D; effective length, 52.8 cm; total length, 60 cm) and 25 kV (reverse polarity) were used for separation. Samples injected into capillary by applying pressure 50 mbar for 6 s. CE operation and collecting the peak data were performed with using 3D DAx software. Capillary tube was rinsed with 0.1 mol L^{-1} NaOH for 5 min, deionized water for 5 min and NBGE for 10 min prior to sample analysis. A constant voltage of - 28 kV was applied for separation and NBGE vial was replaced after 5 runs.

2.5. SPM analysis

SPM analysis was performed on Rayleigh-UV-2100 spectrophotometer (Beijing Rayleigh Analytical Instrument Corporation, China). Spectra were recorded using 10 mM quartz cell. Elico LI615 pH meter (ELICO ® Ltd., Hyderabad, India) was used for pH measurements.

3. Results and discussion

3.1. Method development

3.1.1. TLC separation

TLC is considered as a simple separation technique. Various type substances have been used in the mobile phase to improve separation of isomers and closely moving compounds including native and modified cyclodextrins [20]**.** Initial RP-TLC separations of cis-trans entacapone isomers were performed separately by using either 5-20 $mmol^{-1}$ hydroxylpropyl-β-cycodextrins (HPCD) or heptakis(2,6-di-o-methyl-β-cyclodextrin (DMCD) or α-cyclodextrins (CD) in the mobile phase. The RP-18 F254 , 5 x7.5 cm , aluminium sheets (Merck) plates were used for separations. Cyclodextrin mobile phase additive was prepared in a solvent system consisting of water, acetonitrile, methanol, n-butanol and phosphate buffer of different combination and composition. Nevertheless, none of mobile system was able to resolves the isomers under the tested conditions. Fig.2[A] shows RP-TLC plate image of entacapone developed with solvent system consisting a 5.0 mM phosphate buffer (pH 3.0)-acetonitrile–n-butanol (5:3:1, v/v) and 20 $mmol^{-1}$ hydroxyl propyl-β-cyclodextrin. Considering the high cost of RP-TLC plates and CDs, it was decided to carry out quantitative determination of entacapone in tablet dosage form on normal phase plates (silica gel F254). Mobile phase consisting of toluene, ethylacetate, methanol, acetic acid, and formic acids with different combination and composition were tested for separation of entacapone on normal phase silica gel plates. All the tested conditions, it was observed that entacapone is moving with tailing because due to a strong hydrogen bonding (2-OH, 1-NO_2) of entacapone with Si-OH group. Normal phase TLC plate developed with a solvent system consisting a mixture of toluene-ethyl acetate-methanol- acetic acid (5:3:4:0.5, v/v) is shown in Fig.2[B]. From the figure, it can be seen that the entacapone moved with tailing (Rf 0.8) under the developed condition. It also interesting to note that concentration dependent migration rate of entacapone while developing the plate with a mobile phase consisting of toluene-ethyl acetate-methanol-acetic acid (5:3:4:05, v/v). Fig.2[C] shows the video image of the plate and the amount (ng/spot) entacapone applied on the plate were 100, 200, 400, 600,700 and 800 ng $spot^{-1}$; and the Rf values were 0.20, 0.40, 0.52, 0.60, 0.72 and 0.83, respectively. As can be deduced from the figure, concentration influences the rate (distance) of migration. While increasing the concentration of entacapone, the rate of migration increases and the reason would be, at higher concentration, a weak adsorption of entacapone on silica gel plate. In TLC separation, there is a competitive interaction between solute, solvent and adsorbent. Entacapone is known for inter and intramolecular hydrogen bonding [21]**.** An increase the concentration,

probably the intermolecular hydrogen more favourable and thereof relatively reduce interaction with adsorbent and moved relatively faster. Feigenbaum [22] demonstrated that the adsorption and retention behaviour of nitrophenols had modified by using tetrahydrofuran (THF) in the mobile phase. Keeping in view, further trails were performed with different composition of THF and toluene. A good, compact and baseline separation was achieved with use of THF alone and the condition was optimized for the determination entacapone in tablets.

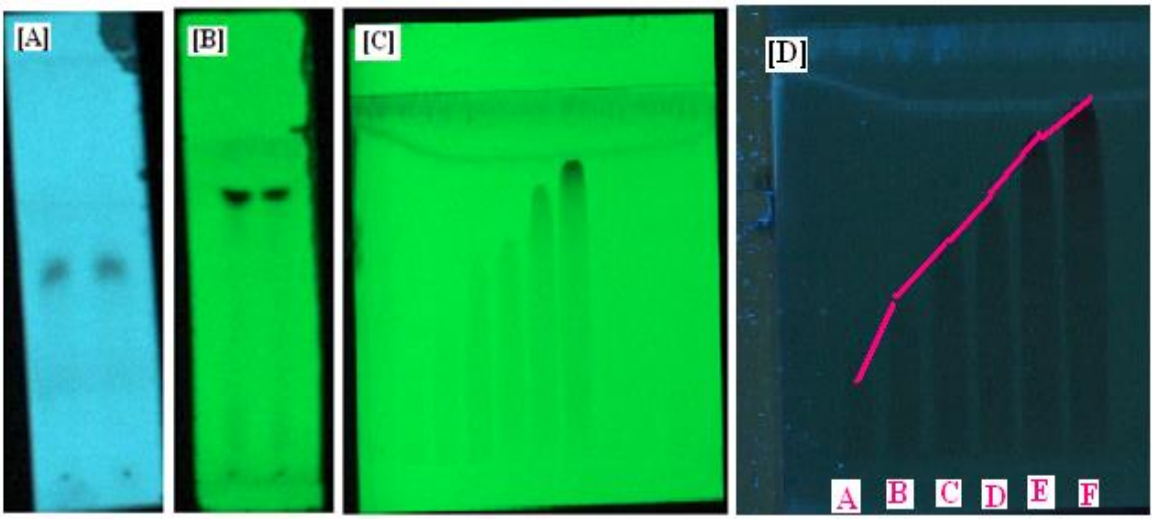

Fig.2. [A] Entacapone (cis-trans) isomers separation on RP-HPTLC plate, developed with 5.0 mM phosphate buffer (pH 3.0)-acetonitrile-n-butanol (5:3:1,v/v) + 20 mM hydroxyl propyl-β-cyclodextrin; **[B]** On silica gel F254 plate, developed with toluene-ethyl acetate-methanol-acetic acid (5:3:4:05, v/v). The video images of the concentration dependent migration of entacapone recorded at UV light 254 nm and florescence 360 nm are shown in **[C]** and **[D]**, respectively and the plates developed with solvent mixture consisting of toluene-ethyl acetate-methanol- acetic acid (5:3:4:05, v/v).

3.1.2. CE separation

Entacapone has a pKa value of 4.5 and become anionic form above pH 5.5. Initial trails on separations were carried out with the most commonly using aqueous background electrolyte (BGE) for the separation of structurally related compounds and isomers. Electropherograms were obtained under different BGEs are given in Fig.3[A]-3[E]. 15 mM tris buffer in 20% methanol (pH 7.5) is shown in Fig.3[A]. Under the condition, no separation was occurred. A electropherogram run with 20 $mmol^{-1}$ hydroxyl propyl-β-cyclodextrin, a chiral additive, in a mixture consisting of 15 $mmol^{-1}$ tris buffer and 20% methanol (pH 8.0) is depicted in Fig.3[B] and under the condition both the isomers were co-migrated and peak broadening was observed. Capillary zone electrophoresis (CZE) performed with 20 mM copper(II) sulphate and 10.0 mM boric acid and the pH 10.0 adjusted with ammonia is shown in Fig.3[C]. Ligand exchange capillary electrophoresis (LECE) carried out with 10 $mmol^{-1}$ copper(II) sulphate and 20 $mmol^{-1}$ hydroxyproline (pH 4.5) and micellar electrokinetic capillary electrophoresis (MEKC) performed with 15.0 $mmol^{-1}$ STB, 12.5 $mmol^{-1}$ boric acid and 20 $mmol^{-1}$ SDS (pH 9.5) are shown in Fig.3[D] and Fig.3[E], respectively. All the separation conditions, either partial separation or peak broadening or no separation were observed. All the separations were carried out using 75 µm I.D x 360 µm) O.D uncoated fused silica capillary tube and a total length of tube was 60 cm and detection was set at 305 nm.

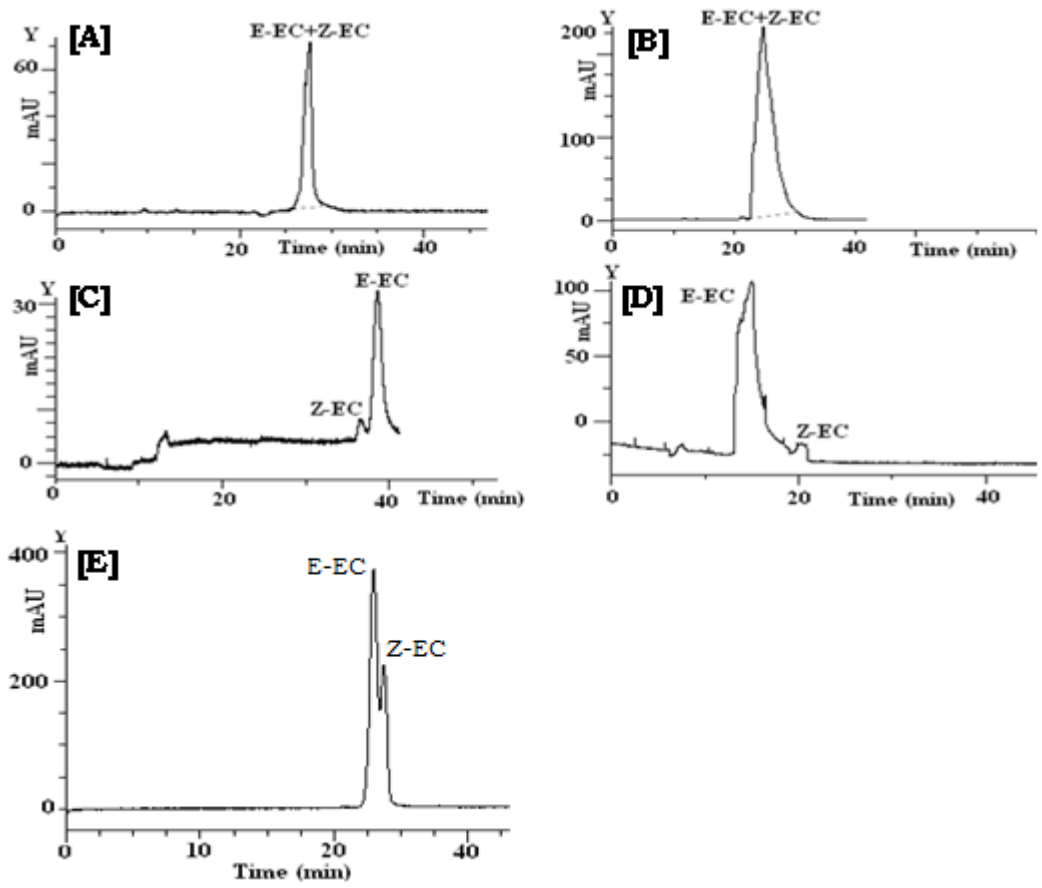

Fig.3.Electropherograms shows the separation behaviour of cis (Z-EC) and trans (E-EC) entacapone isomers at different BGE conditions. **[A]:**15 $mmol^{-1}$ tris buffer 20% methanol (pH 7.5). **[B] :** 15 $mmol^{-1}$ tris buffer, 20 $mmol^{-1}$ hydroxyl propyl-β-cyclodextrin in 20% methanol (pH 8.0). **[C]:** 20 $mmol^{-1}$ copper(II) sulphate ,10 $mmol^{-1}$ boric acid, pH10.0 adjusted with ammonia. **[D]:** 10 $mmol^{-1}$ copper(II) sulphate and 20 $mmol^{-1}$ hydroxyproline, pH 4.5. **[E] :** 15 $mmol^{-1}$ STB, 12.5 $mmol^{-1}$ boric acid , 20 $mmol^{-1}$ SDS (pH 9.5).

Non-aqueous capillary electrophoresis (NACE) is an efficient technique for the analysis hydrophobic and structurally similar compounds [23]. It has several advantages, such as to increase solubility of hydrophobic compounds, improve the separation, low Joule heating effect and low adsorption of analytes on capillary wall. To achieve better separation entacapone isomers, different concentration of STB in different volume ratio of 0.1 NaOH, methanol and 1-propanol were tested. Goods resolution was achieved by using a NBGE consisting a mixture of consisting a mixture of 14.0 $mmol^{-1}$ STB, 7.4% water, 55.6% methanol and 37.0% 1-propanol (*pH 10.5).

Selection of suitable internal standard (IS) is necessary in order to compensate the injection errors in CE. Salicylic acid, 4-hydroxybenzaldehyde, vanillin, vanillic acid and 4-aminobenzoic acid (4-AB) were screened. Among them, 4-AB was found to be suitable as it is well separated from entacapone isomers, sharp peak and baseline separation, and provided good accuracy and precision. Calibration curves were constructed by plotting corrected peak area (peak area/migration time) of the entacapone to that of IS *verses* entacapone concentration.

Typical electropherogram, *in-situ* UV-Vis spectra and contour plot of entacapone isomers obtained under the optimized NBGE are shown in Fig.4 a, b and c, respectively. From the electropherogram and contour plot, it can be seen good separation of entacapone

isomers. In NBGE, selectivity and resolution can be easily control by varying the composition of electrolyte and organic solvents [24, 25]. The UV absorbance spectra of both isomers have almost similar spectral characteristics.

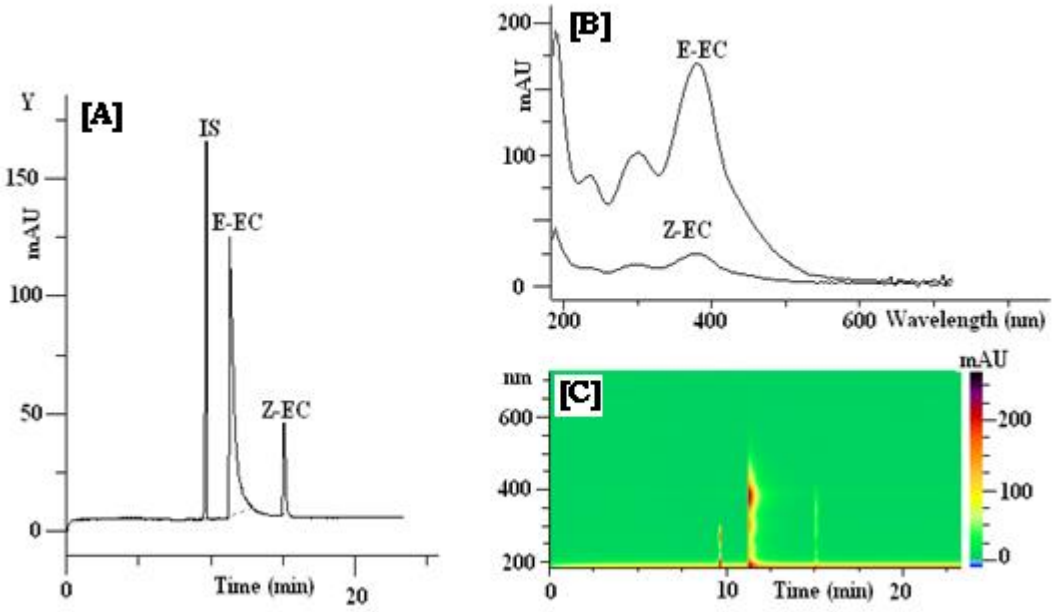

Fig.4. [A] CE separation of cis (Z-EC) and trans (E-EC) isomers entacapone under the optimized NBGE condition. **[B]** In-sito UV-Vis spectra of entacapone isomers and **[C]**, contour plot shows separation of internal standard IS, E-EC and Z-EC. Separation Conditions: 14.0 $mmol^{-1}$ STB in 7.4% water, 55.6% methanol and 37.0% 1-propanol (* pH 10.5) ; 75 µm I.D x 360 µm) O.D uncoated fused silica capillary tube (the total length,60 cm) and detection at 264 nm. Internal standard (IS), 4-Aminobenzoic acid

3.1.3. SPM method

The absorbance spectra of entacapone (3.5 µg mL^{-1}) recorded at different conditions are shown in Fig.5[A] and 5[B]. Fig.5 [A] shows the absorbance spectra recorded in : a) 0.1N HCl, b) 0.1 N NaOH, c) methanol and d) water. The absorbance maxima (λ max) and o.d (in parenthesis) observed for entacapone recorded in 0.1N HCl, 0.1N NaOH, methanol and water were: 305 (0.4367), 379 (0.5394), 384 (0.4586) and 376 (0.1669), respectively. A recovery study of entacapone in tablet dosage form was performed with 0.1 HCl, 0.1 NaOH, methanol, NBGE and NAES. Fig. 5[B] shows, the absorbance spectra recorded in: a)NBGE and b)NAES systems and the molar extinction coefficients calculated for NBGE and NAES were 13808 and 17868 M^{-1} cm^{-1}, respectively. Among the solvent system, better recovery (> 99.5%) was observed with NAES and was optimized for extraction of entacapone in tablet dosage forms.

3.1.4. Linearity, LOD and LOQ

The linearity tested range, regression equation, correlation co-efficient (R^2) and limit of detection (LOD) and quantification (LOQ) of the proposed CE, HPTLC and SPM are given in Table.1. A six-point calibration curve for each method was constructed by plotting analyte peak area (for CE corrected peak of the analyte to that of IS and for SPM O.D) against analyte concentration. All methods were shown a correlation coefficient greater than 0.999. The LOD and LOQ for CE, HPTLC and SPM were 1.52 and 5.02 µg/mL, 8.34 and 24.4 ng $spot^{-1}$, and 0.53 and 1.60 µg mL^{-1}, respectively.

Table. 1 Linearity, LOD and LOQ for the proposed methods.

Method	Linearity range*	Regression equation	R^2	LOD	LOQ
CE	5 -150	y=0.016472x-0.00044163	0.9993	1.52	5.02
HPTLC	50-1500	y=8.246048x-31.6975660	0.9994	8.34	24.3
SPM	1.5-15.0	y=0.0591476x-0.0071538	0.9996	0.53	1.60

* Concentration for spectrophotometric (SPM) and capillary electrophoresis (CE), μg mL^{-1} and for high performance liquid chromatography (HPTLC), ng $spot^{-1}$

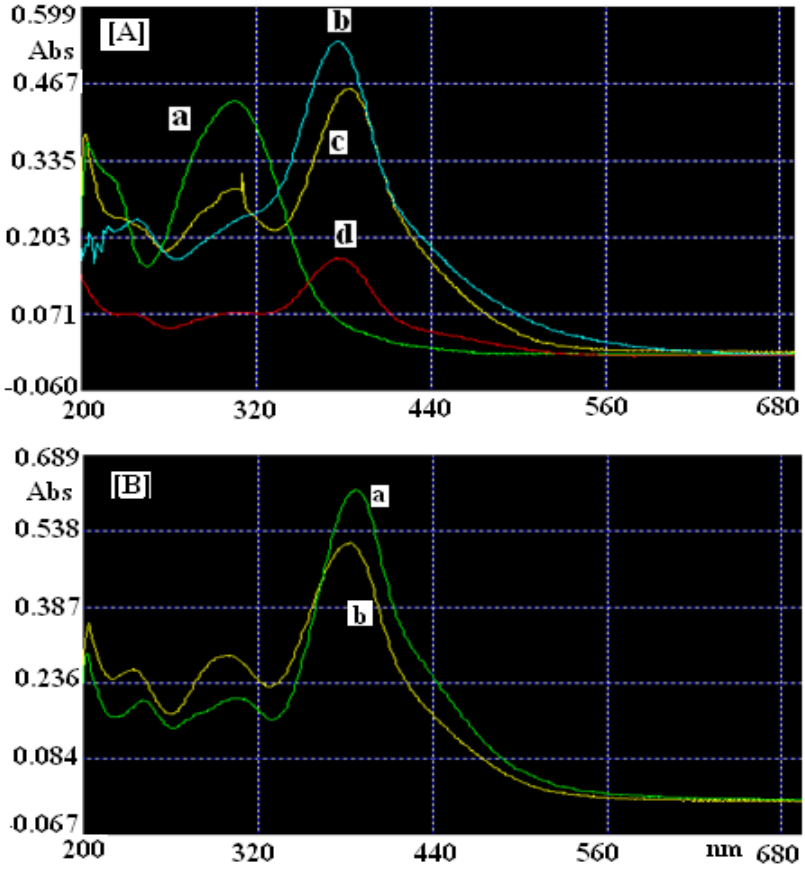

Fig.5 UV spectra of entacapone at different conditions. **[A]:** a) 0.1N HCl, b) 0.1N NaOH C) methanol and d) water. **[B]:** a) NAES and b) NBGE solutions.

3.2. Extraction

3.2.1. Urine

Recent years, UAE technique is gaining importance due to its simplicity, fastness, efficient, cost effective and use of low volume of extractant. During ultrasonication, the ultra sound waves disperse the immiscible organic solvent droplets into the aqueous phase. The phenomenon favours transfer of analyte from aqueous phase to organic phase and extraction efficiency increased [16-18]**.** By using the phenomenon, a trace amount of 2, 4, 6-trichloroanisole was extracted from wine samples [19].

In UAE, several variables such as nature of extraction solvent, salt (sodium chloride) concentration, and pH are significantly affect the extraction. The variables were optimized by modifying each parameter at a time while keeping other parameters constant. Ten milliliter of

blank urine, a constant amount entacapone 5.0 µg/ml was used to perform the recovery studies. Initially, the extraction of entacapone was tested with neutral, acidic (pH 2.0-5.0) and basic (pH 8.0-10) pH conditions. Fig.6 [A] shows the UAE extraction behaviour of entacapone at neutral, basic and acidic condtions.From the Fig.6[A], it can be seen that entacapone was not extractable in organic phase (chloroform) at neutral and basic conditions. However, it was extractable at acidic condition.

The effect of salt concentration (0-5%), ultrasoncation duration (5-15 min), and organic solvents (chloroform, carbon tetrachloride, trichloroethane) were also tested to find out best condition for extraction. Extraction efficiency of entacapone isomers at different conditions were evaluated form the peak area ratio to IS. A constant amount IS (50 µg/mL) was used for the studies. Relatively better extraction efficiency was found at pH 3.5, salt (sodium chloride) concentration of 3.75%, ultrasonication time 10 min and using chloroform as extraction phase. Analysis of entacapone isomers under the optimized extraction condition is shown in Fig.6 [B].

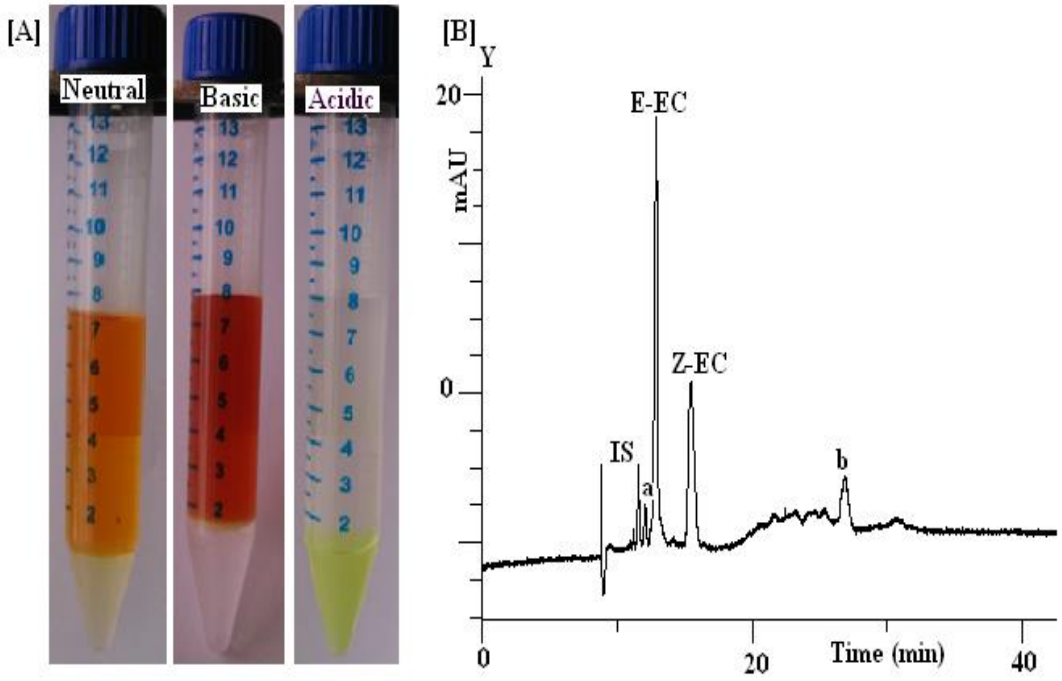

Fig.6 Ultrasonication extraction extraction of entacapone from urine at **[A]** neutral, basic and acidic conations; top layer aqueous and bottom layer chloroform. **[B],** electropherogram shows analysis UAE extraction of entacapone isomers; IS, internal standard (p- aminobenzoic acid) and a) and b) are unknown impurities

3.3. Stability

Stability of entacapone was tested by dissolving 5.0 µg mL^{-1} concentration of solution in NBGE and NAES solutions. The absorbance spectra were recorded at 5 min intervals for a period of 1.0 h. No decrease of the absorbance was observed in NBGE and NAES solutions. The results indicated that the solutions were suitable for SPM and CE analysis.

3.4. Recovery studies

3.4.1. Urine and tablet

Recovery studies of cis and trans isomers of entacapone was performed by spiking three different levels of known amount of each isomer at (5.0, 10.0 and 20.0 µg/mL) in urine and tablet dosage form. The extraction was performed as described procedure in the materials

and method section. The recovery percent and % RSD values are recorded in Table.2**.** From the table it can be seen that the recovery ranges for trans and cis isomers in urine samples were between 94.2 and 97.3 % and % RSD values were between 3.2 and 5.8% by CE method. For the tablets dosage forms, the recovery of entacapone by CE, HPTLC and SPM methods were between 98.2 and 99.3% and the % RSD values were between 0.89 and 1.83%. The recovery percent and RSD% values indicated that the suitability of CE method for the determination of cis and trans isomer in urine and tablet dosage forms. However, the SPM and HPTLC methods were found to be suitable for the determination of single trans isomer in tablet dosage forms.

Table 2 Recovery studies of entacapone urine and tablet samples

Spiked amount ($\mu g\ mL^{-1}$)		CE Urine				Spiked E-EC in Tablets					
						CE		HPTLC		SPM	
		E-EC		Z-EC							
E-EC	Z-EC	%Recovery	%RSD	%Recovery	%RSD	%Recovery*	%RSD	%Recovery	%RSD	%Recovery	%RSD
5.0	5.0	94.2	5.80	95.4	5.21	98.6	1.62	99.7	0.89	99.2	0.95
20.0	10.0	96.2	3.73	97.3	4.23	99.7	1.83	99.3	1.21	99.7	1.05
50	20.0	97.1	3.22	96.8	3.74	98.9	1.42	99.1	1.32	99.8	1.08

E-EC, *trans* entacapone; *Z*-EC, *cis* entacapone. EC=entacapone; SPM, spectrophotometric method

3.5.Analysis of tablets

Table.3 summarizes the content percentage of entacapone in tablet dosage forms by CE, HPTLC and SPM. In CE, percentage of trans and cis entacapone isomers calculated were ranges between 97.23 and 98.35%; and 0.82 and 1.65%, respectively. Nevertheless, the parentage of entacapone content in HPTLC and SPM were ranges between 98.21 and 98.81 and 99.43%; and 98.96 and 99.81%, respectively and the contents were slightly higher than those obtained by CE. This is mainly due to inclusion of cis isomer in the assay by HPTLC and SPM. The % RSD values were < 2.05, 1.35 and 1.06 for CE, HPTLC and SPM, respectively and however, the % RSD values for cis isomer obtained by CE method was relatively higher and were between 1.98 and 2.89%. Fig 7[A] and 7[B] are shows the analysis of entcapone tablet dosage form by CE and HPTLC methods. From the Fig 7[A], it can be seen that the proposed CE method is not only suitable for the selective determination of trans-entacapone but also suitable for detection and determination low level of cis isomer. In a entacapone tablet dosage form (Tablet B), 0.82% of cis isomer was determined and trace level unknown impurities also detected.

Table. 3 Analysis of entacapone in tablet dosage forms by CE, HPTLC and SPM methods

Entacapone (200 mg)	CE (n=6)				HPTLC (n=6)		SPM (n=6)	
	%*E*-EC	%RSD	%*Z*-EC	%RSD	% EC	%RSD	% EC	%RSD
Tablet A	97.23	1.82	1.65	2.08	99.43	0.89	99.81	0.78
Tablet B	98.35	1.34	0.82	2.89	98.81	1.08	99.46	1.06
Tablet C	97.92	2.05	1.05	1.98	98.21	1.35	98.96	0.69

E-EC, *trans* entacapone; *Z*-EC, *cis* entacapone. EC=entacapone; SPM, spectrophotometric method

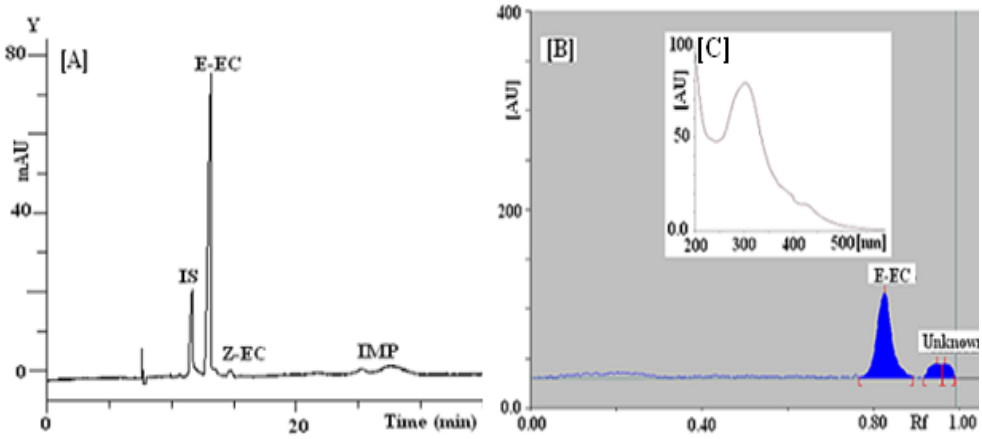

Fig.7.Analysis of entacapone tablet dosage form. [**A]**, analysis of entacapone by CE; IMP= unknown impurities, IS, internal standard (p-aminobenzoic acid). **[B]** shows HPTLC analysis of entacapone In-sito UV spectrum of entacapone (insert) is shown in 7[C].

4. Conclusion

The present ultrasonication assisted extraction (UAE) method for extraction of entacapone in urine sample is simple and efficient. The proposed CE method with non-aqueous background electrolyte (NBGE) is provided better resolution than previously reported aqueous CE methods. The proposed can be couple with MS for detection and determination of unknown impurities in entacapone. Among the three proposed methods (CE, HPTLC, and SPM), SPM is simple and convenient for the determination of entacapone in tablet dosage form. The reported CE and SPM are alternative and improved methods than previously reported methods.

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