ORIGINAL ARTICLE

HPLC-UV DETERMINATION OF INDAPAMIDE IN THE PRESENCE OF ITS MAIN SYNTHESIS AND DEGRADATION IMPURITIES. METHOD VALIDATION

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Abstract

A gradient HPLC with UV detection method was developed for the quantitative determination of indapamide in the presence of 6 of its synthesis and degradation impurities. The separation of analytes was performed on X-Terra, C_{18}, 250 mm × 4.6 mm, 5 µm (Waters) column using a mixture of aqueous Na_2EDTA, acetonitrile and methanol, with detection at 254 nm. The developed method was validated and is suitable for in process purity evaluation of indapamide synthesis, indapamide stability in bulk and in pharmaceutical dosage forms.

Rezumat

A fost elaborată o nouă metodă HPLC cu detecție UV pentru analiza cantitativă a indapamidului în prezența a 6 din impuritățile sale din sinteză și de degradare. Separarea analiților s-a realizat pe o coloană X-Terra, C_{18}, 250 mm × 4,6 mm, 5 µm (Waters) în gradient folosind, un amestec de soluție apoasă de Na_2EDTA, acetonitril și metanol, în timp ce detecția s-a făcut în UV la lungimea de undă de 254 nm. Metoda a fost validată și este aplicabilă pentru evaluarea purității în procesul de sinteză al indapamidului sau la evaluarea stabilității materiei prime și a formelor farmaceutice cu indapamid.

Keywords: indapamide, HPLC, purity, validation

Introduction

Indapamide (IDP) is a "thiazide like" diuretic widely used in the treatment of hypertension alone or in combination with an angiotensin converting enzyme inhibitor [11, 21]. Nowadays there are two main synthesis routes [5, 26, 28] of indapamide (Figure 1). The difference between this two synthesis routes are related to the transformation of 2-methylindoline (MI) in 1-amino-2-methylindoline hydrochloride (CAMI). This transformation can be performed in one step (Figure 1, STEP 1a) by using hydroxylamine-O-sulphonic acid. The same transformation can be performed in two steps (Figure 1, STEP 1b and STEP 1c). In STEP 1a, MI is converted to 2-methyl-1-nitrosoindoline (imp A) by sodium nitrite. STEP 1c consists in the reduction of imp A to CAMI with $LiAlH_4$. The common steps of the two main synthesis routes of indapamide are: the conversion of 4-chloro-3-sulphamoylbenzoic acid (AcCSB) to 4-chloro-3-sulphamoylbenzoyl chloride by means of thyonyl chloride/toluene and the reaction of CAMI with 4-chloro-3-sulphamoylbenzoyl chloride in a mixture of tetrahydrofuran, sodium bicarbonate and water. Considering the two synthesis routes, the synthesis and degradation impurities of indapamide are: AcCSB (starting material or originating from 4-chloro-3-sulphamoylbenzoyl chloride hydrolysis), MI (starting material), imp A (intermediate material, also mentioned by European Pharmacopoeia in the Indapamide Monography), CAMI (intermediate material), N-(4-chlorobenzoyl-3-sulfamoyl)-2-methylindoline (NCSBMI) originating from reaction among 4-chloro-3-sulphamoylbenzoyl chloride and MI that is present as impurity in CAMI and 4-chloro-3-sulfamoyl-n-(2-methyl-1H-indol-1-yl)-benzamide (imp B – originating from IND oxidation, also mentioned by European Pharmacopoeia in the Monography of Indapamide). According to European Pharmacopoeia [3] maximum allowed content of imp B is 0.3%, unspecified impurities 0.1% and total impurities 0.5%.

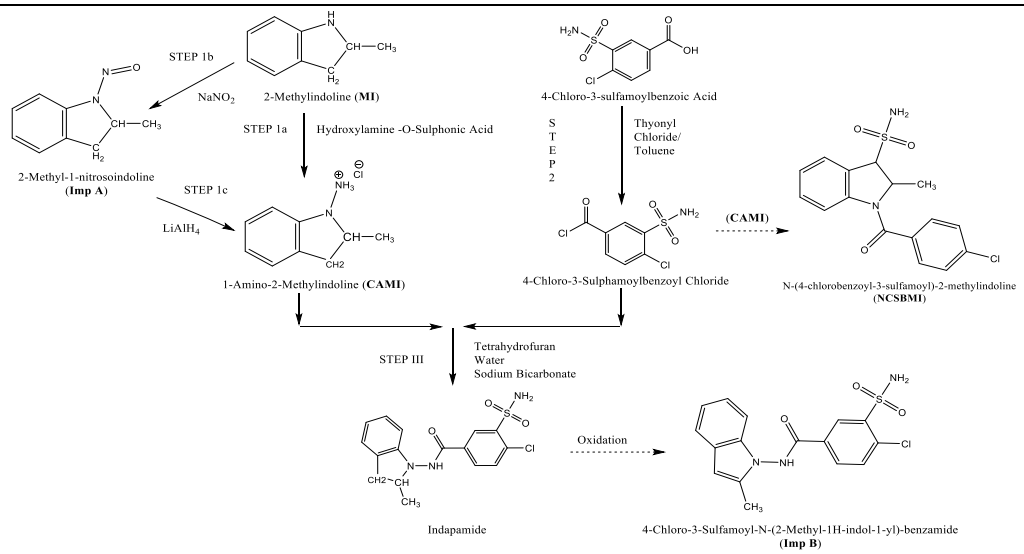

Figure 1.
Indapamide synthesis [5, 26, 28]

Many papers describing indapamide analysis in pharmaceuticals and biological matrices, have been published, some of them being indapamide stability indicating methods: HPLC [3, 7, 8, 13, 14, 16, 18], spectrophotometric [22, 23, 29], spectrofluorimetric [17, 29], TLC [10, 29], capillary electrophoresis [25, 30], voltametric [20], chemiluminiscence based [9] and NIR [19, 27]. None of those papers describes simultaneous determination of indapamide and its synthesis and degradation impurities. The aim of this study was to develop and validate an HPLC method for simultaneous quantification of indapamide and its synthesis and degradation impurities, considering international guidelines and latest protocols [1-4, 6, 12, 15, 18, 24].

Materials and Methods

Materials. Lactose (Tablettose 80, Meggle), microcrystalline cellulose and sodium starch glycolate (JRS Pharma, Germany), colloidal silicon dioxide (Aerosil, Rohm-Pharma Polymers, Germany), polyvinylpyrolidone (BASF, Germany), magnesium stearate (Union Derivan, Spain).
Standards. IDP (Sigma Aldrich, Germany), Imp A and Imp B (European Pharmacopoeia CRS, EU), AcCSB (Sigma Aldrich, Germany), NCSBMI (Mikromol GmbH, Germany), CAMI (Mikromol GmbH, Germany), MI (Sigma Aldrich, Germany).
Reagents. Anhydrous acetic acid and Na_2EDTA (analytical purity, Chimopar, Romania, and Merck, USA, respectively), acetonitrile (ACN, HPLC purity, Sigma Aldrich, Germany), methanol (MeOH, HPLC purity, Merck, USA), ultrapure water (Conductivity < 1.2 µS/cm, Millipore Q).
Equipment. Analytical balance (readability 0.01 mg, Mettler Toledo, USA), water purification system Millipore Q (Millipore, USA), automatic pipettes (10 - 100 µL, 100 - 1000 µL, Eppendorf Research), A class volumetric flasks (5 and 10 mL, Schott Duran, Germany), 2 mL Eppendorf tubes, acrodisc filters (13 mm, 0.45 µm, syringe filters), HPLC system (Waters Alliance 2695, quaternary pump, column thermostat, autosampler, sample thermostat, degasser, DAD Waters 996 detector).
Software: Empower 2.0 chromatography software (Waters), Excel 2003 (Microsoft).
Chromatographic conditions: Column: X-Terra, C_{18}, 250 mm × 4.6 mm, particle size 5 µm (Waters).
The mobile phase (v/v) and the gradient program are depicted in Table I.

Table I
Mobile phase (v/v) and gradient program

Time (min)	%A	%B	%C	Flow rate (mL/min)	Mobile phase
0	65	17.5	17.5	1.2	A – Na_2EDTA 0.2 g/L + anhydrous acetic acid 0.1 mL/L
2.5	65	17.5	17.5	1.2	B – acetonitrile
6	70	15	15	1.2	C – methanol
9	72	14	14	1.3	
16	72	14	14	1.3	
16.5	65	17.5	17.5	1.3	
20	62	19	19	1.3	
25	65	17.5	17.5	1.3	
28	65	17.5	17.5	1.2	

Injection volume: 10 µL, column temperature: 40°C, sample temperature: 4°C and detection wavelength 254 nm.

Stock solutions and working solutions

Diluent: was prepared by mixing ACN and MeOH 1:1 (v/v).

IDP standard stock solution (2.0062 mg/mL): was prepared by weighing appropriate amount of IDP standard and dissolving with the diluent in order to obtain 5 mL solution. This solution was kept at 4°C, protected from light.

IDP standard working solutions: 5 solutions (range of concentration: 0.803 - 1.207 mg/mL) were prepared extemporaneous by diluting IDP standard stock solution with the initial mobile phase.

Impurities standard stock solutions (Imp. A 1.082 mg/mL; Imp.B 1.000 mg/mL; MI 1.02 mg/mL; CAMI 1.058 mg/mL; NCSBMI 1.042 mg/mL; AcCSB 1.088 mg/mL): were prepared by weighing appropriate amounts of standards and dissolving them in diluent in 5 mL volumetric flasks. These solutions were kept at 4°C, protected from light.

IDP and impurities standard working solutions: 5 solutions *(*concentration ranges: IDP 0.803 - 1.207 mg/mL; Imp. A 0.541 - 8.656 µg/mL; Imp. B 0.521 - 8.336 µg/mL; MI 0.512 - 8.184 µg/mL; CAMI 0.516 - 8.256 µg/mL; NCSBMI 0.500 - 8.000 µg/mL; AcCSB 0.544 - 8.704 µg/mL) were prepared extemporaneous by diluting IDP standard stock solution and impurities standard stock solutions with the initial mobile phase.

Excipients blank: was prepared by mixing, for 5 minutes, appropriate amounts of lactose, micro-crystalline cellulose, sodium starch glycolate, colloidal silicon dioxide, polyvinylpyrolidone, magnesium stearate using a planetary mixer as previously published. Appropriate amount of excipients mixture was shook with diluent in a volumetric flask and filtered through acrodisc filters. An aliquot of the filtrate was diluted with the initial mobile phase.

Methods

Validation protocol

System suitability: in order to validate system suitability, the following parameters and critical limits were considered: resolution (R_s) > 1.5 (calculated as stated in European Pharmacopeia); tailing factor (T) < 2; theoretical plates (N) > 2000; for 5 injections RSD < 2%.

Specificity: by separately injecting IDP and impurities standard working solutions and excipients blank the obtained chromatograms must prove no interferences.

Linearity

Linearity was tested for IDP and 6 impurities and was proved at five concentration levels (k = 5) in triplicate (n = 3). Calibration curves were plotted by linear regression both for IDP and impurities standard working solutions. Correlation coefficient (r) was above 0.99. Also for linearity validation the following statistical tests were performed.

Statistical analysis of linearity: Cochrane test (variances homogeneity evaluation), Fisher test (regression curves validity, slope significance evaluation), t Student test (for intercept comparison with null), considering a 5% error probability for all k = 5 concentration levels.

Detection (LOD) and quantification limit (LOQ) were determined based on signal-to-noise approach (S/N).

$$LOD = 3 \times (S/N) \text{ and } LOQ = 10 \times (S/N)$$

Precision

In order to prove method precision the following parameters were evaluated: repeatability or the precision on the same day and intermediate precision or precision in different days (reproducibility). Analyses were performed on a single concentration level (100%) for IDP and impurities, 6 replicates per day in three different days. For linearity validation, the following statistical tests were performed.

Statistical analysis of precision: Cochrane test (variances homogeneity evaluation), repeatability variation coefficient (CV_r%) and reproducibility variation coefficient (CV_R%), considering a 5% error probability and 18 (6 × 3) samples.

Accuracy

Accuracy was estimated by means of recovery using calibration curves for IDP and impurities (constructed as in linearity protocol, five concentration levels and three replicates). For accuracy validation, the following statistical tests were performed.

Statistical analysis of accuracy: Cochrane test (intra-group variance evaluation), Fisher test (mean recovery validity), t Student test (confidence interval for mean recovery I_{mr}), considering a 5% error probability.

All statistical tests presented in this protocol were performed using Microsoft Excel® 2003.

Results and Discussion

System suitability

The calculated values of the mentioned parameters in the validation protocol are presented in Table II.

Table II

System suitability parameters

	AcCSB	MI	CAMI	Imp A	IDP	NCSBMI	Imp B
R_s	2.739	8.676	24.342	2.912	9.785	5.180	
T	1.105	1.072	1.094	0.984	0.985	1.012	1.041
N	7405.22	9234.68	11985.22	9722.67	7144.45	52898.44	33198.92
RSD (%)	0.781	0.823	0.645	0.982	1.401	1.033	0.756

IDP and impurities showed good resolution (R_s ranging 2.739 - 24.342), symmetric peaks (T ranging 0.984 - 1.105), good efficacy (N ranging 7405.22 - 52898.44) and also good injection repeatability (RSD ranging 0.645 - 1.401). All system suitability parameters took into consideration fulfilled the pharmacopoeial requests.

Specificity

By injecting IND and impurities standard working solutions the following retention times (in minutes) were observed: AcCSB - 3.465; MI - 3.904; CAMI - 5.472; Imp A - 15.533; IDP - 16.920; NCSBMI - 22.769 and Imp B - 25.229. By injecting excipients blank no interference was observed (Figure 2).

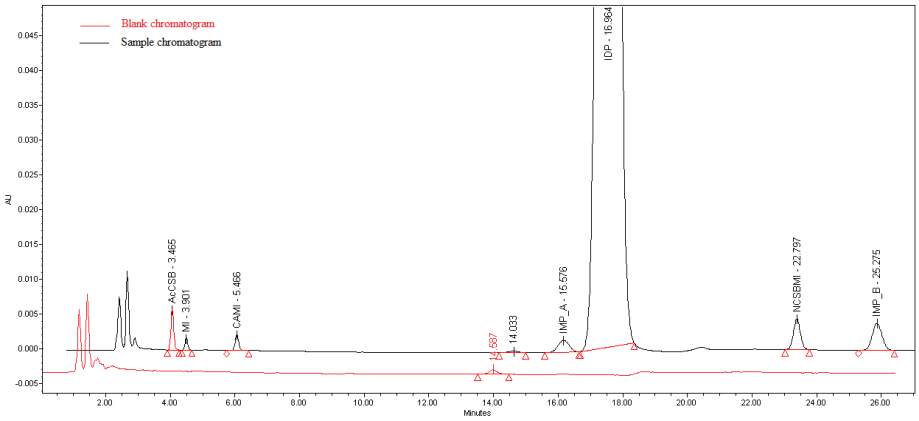

Figure 2.
Overlaid chromatograms of sample and blank

The results demonstrate that the analytical procedure is specific for these particular analytes.

Linearity

Calibration curves parameters and range are presented in Table III.

Table III

Calibration curves parameters and range

Analyte	Slope (a)	Intercept (b)	Correlation coefficient r	Range (µg/mL)
IDP	14304194.988	-54795.395	0.9961	803 - 1207
IMP A	8969.457	-253.486	0.9997	0.541 - 8.656
IMP B	16217.004	12569.500	0.9995	0.521 - 8.336
MI	2487.468	205.778	0.9997	0.512 - 8.184
CAMI	4309.234	616.500	0.9982	0.516 - 8.256
NCSBMI	15870.902	1289.069	0.9996	0.500 - 8.000
AcCSB	8000.490	327.630	0.9998	0.544 - 8.704

As presented in Table III, correlation coefficients of all calibration curves were higher than 0.996.

Statistical treatment of linearity data is presented in Table IV. Variances are homogeneous, all $C_{calc.}$ fulfil Cohran test ($C_{calc.} < C_{theor.}$). Regression curves are valid, all $F_{calc.}$ are lower than $F_{theor.}$. Since all $F_{calc.}$ exceed $F_{theor.}$ prove that all calibration curves have significant slopes. All calculated values ($t_{calc.}$) are below $t_{theor.}$, therefore we can affirm that intercept for all calibration curves significantly differs from 0.

Table IV

Statistical evaluation of linearity

Analyte	Variances homogeneity (Cochrane test)	Regression curves validity (Fischer test)	Significant slope (Fischer test)	Intercept comparison with null (t -Student test)
	Calculated values			
IDP	0.376	5.44×10^{-7}	853.62	9.35×10^{-9}
IMP A	0.433	0.133	21139.85	2.54×10^{-6}
IMP B	0.376	0.020	14392.65	4.00×10^{-5}
MI	0.529	0.533	25389.10	2.84×10^{-5}
CAMI	0.528	0.400	3516.44	2.80×10^{-5}
NCSBMI	0.376	0.023	14457.22	4.46×10^{-6}
AcCSB	0.646	0.300	42312.41	4.11×10^{-6}
	Theoretical values			
	0.68	3.71	4.67	2.160
	Acceptance criteria			
	$C_{calc.} < C_{theor.}$	$F_{calc.} < F_{theor.}$	$F_{calc.} > F_{theor}$	$t_{calc.} < t_{theor.}$

All system linearity parameters considered in validation protocol fulfil the predefined criteria.

Detection (LOD) and quantification limits (LOQ)

Detection and quantification limits calculated based on signal to noise (S/N) are presented in Table V.

Table V

LODs and LOQs

	AcCSB	MI	CAMI	Imp A	IDP	NCSBMI	Imp B
LOD (µg/mL)	0.147	0.135	0.096	0.098	0.057	0.129	0.068
LOQ (µg/mL)	0.049	0.450	0.320	0.326	0.190	0.431	0.227

Precision

As stated in Table VI, all variances are homogeneous (since all $C_{calc.} < C_{theor.}$), and all repeatability and reproducibility coefficients are below 2%. There is a single exception, CAMI, in which case $CV_r\%$ = 2.59% and CVR% = 4.83%, but since Cohran test has been passed, we consider that the method is precise.

Table VI

Statistical evaluation of precision

Analyte	Variances homogeneity (Cochrane test)	Repeatability variation coefficient (CV_r %)	Reproducibility variation coefficient (CV_R %)
	Calculated values		
IDP	0.39	1.10	1.11
IMP A	0.38	1.93	2.00
IMP B	0.44	1.47	1.63
MI	0.52	1.90	1.97
CAMI	0.47	2.59	4.83
NCSBMI	0.59	1.01	1.40
AcCSB	0.54	1.01	1.02
	Theoretical values		
	0.68	-	-
	Acceptance criteria		
	$C_{calc.} < C_{theor.}$	-	-

Accuracy

Statistic evaluation of accuracy is presented in Table VII. All variances are homogeneous ($C_{calc.} < C_{theor.}$), medium recoveries are valid ($F_{calc.} < F_{theor.}$) and all confidence intervals for the medium recovery are closed to 100%.

Table VII

Statistical evaluation of accuracy

Analyte	Intragroup variance homogeneity (Cochrane test)	Medium recovery validity (Fischer test)	Confidence interval of the mean recovery (%)
	Calculated values		
IDP	0.65	0.64	99.266 - 100.735
IMP A	0.61	0.35	96.995 - 100.879
IMP B	0.58	0.01	94.101 - 102.927
MI	0.61	0.27	95.396 - 102.124
CAMI	0.62	3.21	96.926 - 104.309
NCSBMI	0.48	0.08	94.164 - 101.114
AcCSB	0.50	0.04	96.935 - 100.877
	Theoretical values		
	0.68	3.48	-
	Acceptance criteria		
	$C_{calc.} < C_{theor.}$	$F_{calc.} < F_{theor.}$	-

Conclusions

A HPLC-UV method for simultaneous quantitative determination of indapamide and its synthesis and degradation impurities was developed. The method was validated in terms of system suitability, specificity, linearity, range, precision and accuracy. LODs and LOQs were determined. The developed method is suitable for in process control of indapamide synthesis. Also, the method is suitable for stability studies of indapamide in bulk and in tablets.

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