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DEVELOPMENT AND VALIDATION OF METHOD OF ANALYSIS FOR A PROPOSED COMBINATION OF AMOXICILLIN AND IBUPROFEN IN ORAL DOSAGE FORM USING HPLC

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ABSTRACT

The aim of this work is to develop a simple**, validated method of analysis** using HPLC to be used to measure amount of amoxicillin (AM) and ibuprofen (IB) simultaneously in oral tablets. The method was developed successfully and gave a very good separation of both compounds. The parameters of validation like linearity, precision, accuracy, system suitability were all within specification of EMA. The method was used in evaluation of drug content and dissolution of amoxicillin and ibuprofen **from prepared oro-dispersible tablets** contain 100 mg ibuprofen and 125 mg amoxicillin for pediatric use.

INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry that involves identification, determination, quantification and purification of a substance. Also, separation of the components of a solution or mixture, or determination of structure of chemical compounds (El-Yazbiet al., 1999). The substance may be a single compound or a mixture of compounds and it may be in any type of the dosage forms (Kolhe et al.,2013, Kawalec et al., 2018). Pharmaceutical preparations contain different types of substances; this makes accurate measurement especially of API(s) very critical for evaluation of the dosage form (KwonandLee,2010). Analytical determination is based on the measurement of one or more physical, chemical or structural property which is related directly or indirectly to the amount of constituent present in the sample (Ding et al.,2018, Ren et al.,2012). Pharmaceuticals are chemicals mostly of organic, originwhich have some propertiesto be used in qualitative and quantitative measurement (Akshatha et al., 2018, D'Atriet al.,2018). To select a proper analytical method, some criteria are to be taken in consideration, like ; nature of analyte, concentration range in work, possible interference with other materials, time required for analysis and facilities available (Raza et al.,2017, EMA, 2018). Fixed combination therapy is gaining a lot of attention in pharmaceutical industry (Bell, 2013). It serves many advantages in achieving different therapeutic paradigms (Yogendra et al., 2016) However many challenges are to be overcome, among these evaluation of the

product in minimum time and high level of accuracy (Asciertoand Marincola, 2011)**.** Development of suitable method of analysis to measure double or triple API in a pharmaceutical preparation has been highly developed with the development of technology of analysis (Pal et al., 2013). However, high level of accuracy and precision is always required (Chundawat et al.,2011).

The aim of this work is to develop method of analysis of a combination of amoxicillin (AM) and ibuprofen (IB) to be used in evaluation of a proposed combination of these two API in a pediatric oral dosage form contains 125 mg AM and 100 mg IBas ODT using HPLC and according to the specification of EMA.

METHODS

Materials: HPLC grade solvents, API and all tablet additives were kindly gifted by Hikma® Pharmaceutics and Dar Al-Dawa®Pharmaceutics in Jordan.

Development and validation of HPLC method for analysis of AM and IB: High performance liquid chromatography (HPLC) system was used in development and validation of a method that detect qualitatively and quantitatively both AM and IB concomitantly.An HPLC (Finnigan Surveyor) was used in this study and it composes of the following: ChromQuest software 4.2.34 Solvent delivery systems pump (LC Pump Plus), autosampler Plus, UV-VIS Plus Detector, Hypersil Thermo Electron Corporation, BDS C-18 Column (150 mm x 4.6 mm, 5µm) and computer System, Windows XP, SP3.

Chromatographic conditions and Method parameters: Table (1) below shows the chromatographic conditions and instrument settings. Wavelength was chosen to suit both API(s) after trials.

Preparation of standard solutions: In all preparations, 50 mg of AM and 20 mg of IB were taken in a 100-mL volumetric flask, and about 50 mL diluting solution (mobile phase) was added and sonicated for five minutes to dissolve both properly. Then volume was made up to the mark with the same diluent. This was stock solution. Suitable dilutions by same solvent were made to obtain target concentration for each parameter of validation.

Method validation: The method was validated according to the EMA guideline with respect to linearity and range, precision (repeatability and reproducibility, inter-day and intra-day), solution stabilityaccuracy and system suitability (Shabir et al., 2013).

Linearity and range:

Linearity was checked onfive different concentrations within 0.1-0.35 mg/ml for AM and 0.1-0.32 mg/ml for IB of the nominal standard concentration. The linearity of the proposed method was evaluated by using calibration curve to calculate coefficient of correlation, slope, and intercept values. The range of analytical procedure is the intervalbetween upper and lower concentration in the analyte in the sample for which it was demonstrated that the analytical procedures was suitable.

Precision

The precision is used to ascertain analysis repeatability by evaluating number of samples containing known amount of analyte. The precision of the assay was assessed with respect to repeatability and reproducibility. The precision of the proposed method was checked by repeatability and system suitability, intra- and inter-day repeatability of responses after replicate injections and expressed as %RSD (percent relative standard deviation). For each active ingredient

10samples were analyzed for repeatability and system suitability under same operating conditions and for the same solution. Target concentration was equal to 0.25 mg/ml for AM and 0.20 mg/ml for IB. For inter-day precision a solution of 0.2 mg/ml of AM and 0.16 mg/ml for IB were prepared and readings were taken at time zero and after 24 hours. Intera- day precision was performed over 48 hours using the same concentrations above and all10 samples were analyzed at time zero and 48 hours. Each time 3 replicates were taken and average area under the curve (AUC), standard deviation (SD) and percent relative standard deviation(% RSD) were calculated.

Solution stability: Stability of drugs in diluting solvent and mobile phase was checked by rendering the test solution which contained 25 mg AM +20 mg IB /100 ml in tightly capped vials at room temperature for 48 hours. Dilution 1/100 was made to produce concentrations 0.25 mg/ml AM+0.2 mg/ml IB. Two solutions were prepared, solution 1, the readings were taken at time zero and 24 hours. And for solution 2, readings were taken at time zero, 24 hours and 48 hours. The solutionswere analyzed for both drugs and average AUC, SD and RSD were calculated.

Accuracy: The accuracy of an analytical method expresses the nearness between the expected value and the value found. In the present study, successive analysis (n = 3) for three different concentrations of standard mixture (10 mg AM+10 mg IB/100 ml , 20 mg AM +20 mgIB/100 ml and 30 mg AM+ 30 mg IB/100 ml) was carried out to determine the accuracy of the proposed method. Serial dilutions were made to obtain concentrations within the calibration curves. The accuracy was expressed as % recovery of each substance from the prepared concentration.

System suitability: The purpose of the system suitability test is to ensure that the complete testing system, including instruments, reagents, columns, analysts etc., is adequate for the intended analysis. The following parameters are usually determined: theoretical plate count, tailing factors, and reproducibility.Those parameters were determined for each run.

Evaluation of the prepared formula

In the bulk work an ODT contains combination of 100 mg IB and AM 125 as FCT for pediatric use were prepared successfully. The DOE was used to optimize the formula and the response factors (under publication). This method was used in evaluation of drug content and release and dissolution of the prepared formula.Tests were performed according to the standards of USP of ODT. Drug APIs content of tablets was tested by crushing twenty tablets (theoretically contain 2 g IB and 2.5 g of AM) and an amount of powder corresponding to content of 1 g IB and 1.25 g AM was weighed. APIs were extracted with 60 ml chloroform and 60 ml alcohol in a separatory funnel and shaked for 20 minutes, then layers were separated. The aqueous layer contained AM and the chloroform layer contained the IB. Chloroform layer was evaporated and the residue was dissolved in 50 ml of 95% ethanol. Serial dilutions were made by the mobile phase of the method specified, and amount of IB was calculated. For drug dissolution, apparatus II was chosen and the dissolution media was phosphate buffer pH 6.8 as a compromised point to measure both API(s). Samples drawn in different time intervals and suitable dilutions were made by the mobile phase and measured using the new validated method. Percent dissolution at 30min was chosen to indicate drug release as mentioned in the design of work.

Table 1: Chromatographic conditions

Method parameters	
Stationary phase	C18,250x4.6 mm 5 mμ(Hypersil)
Mobile phase	Solution A: buffer pH5 Solution B:Acetonitril (gradient system)
Wavelength	220 nm
Flow rate	1.0 ml/min
Concentration range	0.1-0.35 mg/ml
Target concentration	0.25 mg/ml
Diluent	Acetonitril: phosphate buffer (50:50)
Injection volume	20 µl
Column oven	25C^{o}
Tray Temperature	15 C^{o}

Table 2: Results of Precision Test of AM

Test	Target concentration	Average AUC (n=10)	SD	%RSD
Repeatability and system suitability	0.25 mg/ml	5239250	42934	0.819
Intraday precision	0.2 mg/ml	4246172	4.6661.23	0.91
Inter-day precision	0.2 mg/ml	4216557	58268.4	1.36

Table 3: Results of Precision Test of IB

Test	Target concentration	Average AUC (n=10)	SD	%RSD
Repeatability and system suitability	0.2 mg/ml	9452000	23630	0.25
Intraday precision	0.16 mg/ml	7771187	50512.72	0.65
Inter-day precision	0.16 mg/ml	7858227	86.440.5	1.1

Table 4: Results of Solution Stability of AM for 24 and 48 Hours at Room Temperature.

Solution #	Amoxicillin (prepared conc.=0.25mg/ml)		Ibuprofen (prepared conc.=0.20mg/ml)	
	Solution 1(24 hr) N=3	Solution 2(48 hr) N=3	Solution 1(24 hr) N=3	Solution 2(48 hr) N=3
Average AUC Initial reading (time 0)	5163841	5301931	9411179	9440202
Average AUC (24 or 48 hour reading)	5301931	5227649	9439237	9425306
SD	97644	87183	19840	10533
%RSD	1.866	1.648	0.210	0.112
Percent from initial concentration	100.6	101.0	100.1	99.7

Table 5: Accuracy Results of AM and IB.

AM	Conc. prepared	Amount dissolved (mg)	Amount recovered(mg)	% Recovery	RSD%
Solution 1(n=3)	0.1 mg/ml	10.00	10.12	101.2%	0.74
Solution 2(n=3)	0.2 mg/ml	20.00	19.94	99.7 %	0.85
Solution 3(n=3)	0.3 mg/ml	30.00	30.18	100.6 %	1.15
IB					
Solution 1(n=3)	0.1 mg/ml	10.00	10.09	100.9 %	0.97
Solution 2(n=3)	0.2 mg/ml	20.00	20.30	101.5%	1.1
Solution 3(n=3)	0.3 mg/ml	30.00	29.958	99.86%	1.3

Table 6: Results of System Suitability of AM and IB.

	Conc.	Retention time (min)	Area	Average±SD	%RSD	Theoretical plates	Tailing factor
AM	0.1 mg/ml	3.2	2602824	2621740± 16508.86	0.63	781968	1.4
		3.2	2629156			771130	1.6
		3.2	2633241			780500	1.6
	0.35mg/ml	3.2	6982715	6971481± 18158.26	0.26	688707	1.4
			6981196			685203	1.3
			6950532			700923	1.5
IB	0.16 mg/ml	14.6	7781970	7758185± 84359.93	1.08	641935	1.2
		14.6	7828099			635780	1.2
		14.6	7664486			638283	1.2
	0.32 mg/ml	14.7	15020815	14951846± 111104.2	0.74	464371	1.0
		14.7	15011045			460833	1.0
		14.7	14823678			456227	1.0

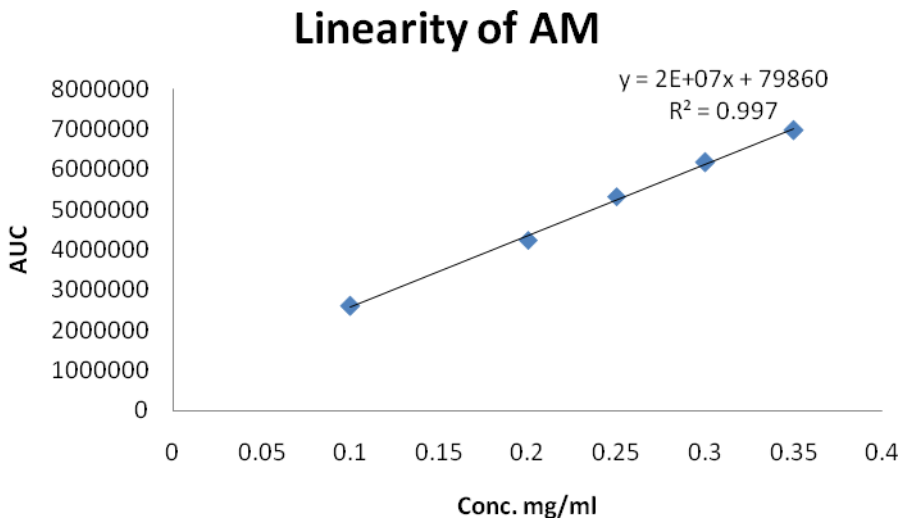

Fig. 1: Calibration curve of AM.

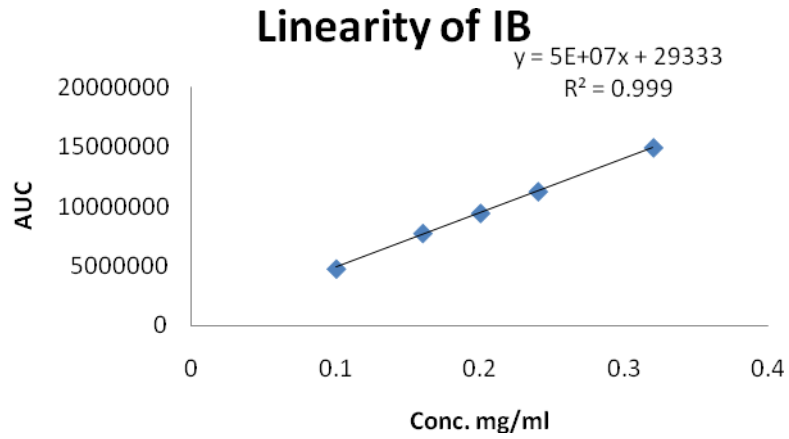

Fig. 2: Calibration curve of AM.

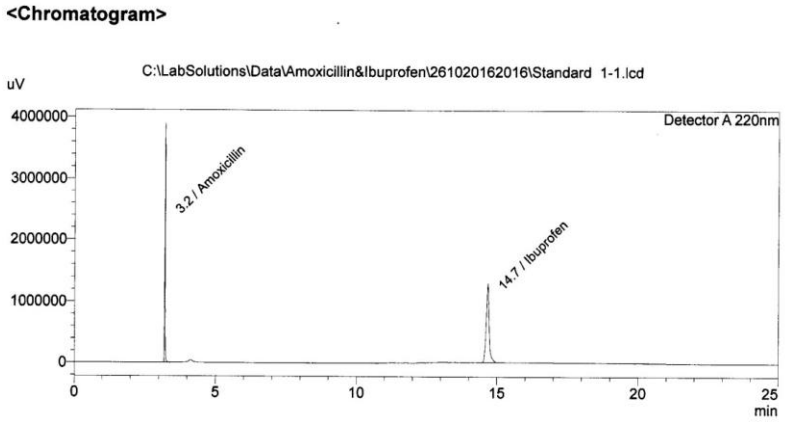

Fig. 3: Chromatogram of standard concentration (0.1 mg/ml AM+0.1 mg/ml)

RESULTS AND DISCUSSION

Method validation

Linearity: According to USP, the correlation coefficient (*R*) for a calibration curve must be >0.995. The correlation coefficient was found to be more than 0.995 for both drugs indicating good linearity of calibration curve. The linearity curves are shown in figure (1) and (2). And chromatograms taken from two samples are shown in figures (1) and(2). Retention time of amoxicillin was equal to 3.2 min. and that of ibuprofen was 14.7 min. the time between the appearance of amoxicillin and ibuprofen was long enough to exclude any interference and overlap between them. The peaks were sharp and clear as shown in figure 1. The range of concentration in all validation parameters was from 0.1 – 0.35 mg/ml AM and IB from 0.1 -0.32 mg/ml. For the precision, test involved repeatability and system suitability, inter-day and intra-day precision. Results shown in tables (2) and (3) show that the RSD in all cases is less than 2%. Results of **solution stability** of AM and IB are expressed in tables 4. The solution contained both drugs was found to be stable in room temperature for 48 hour. This will help to ensure accurate readings during the dissolution test and also give a good indication of drugs compatibility when mixed in single tablet. The **accuracy** results of the developed method for both drugs are illustrated in table 5. The method showed high ability to recover the dissolved materials and the percent recovery and %RSD are all within the guidelines. Selected solutions readings were made to express the results of **system**

suitability. Retention time, peak area, theoretical plates and tailing factor of three levels concentration are shown in table 6.

3.2 Evaluation of the optimized formula

Based on the confirmation of the DOE the optimized formula was prepared and it contained the following ingredients per one tablet as ODT: AM 125 mg, IB 100 mg, mannitol 100 mg, aerosil 4 mg, Na stearate 4 mg, Sodium Starch Glycolate43 mg, Avicel 2.8 mg and sucrose 4.7 mg. A batch of 200 tablets was prepared and evaluated using the developed method for drug content and drug dissolution to be used in the production of the tablets. Drug content of both API(s) was equal to 96±5% for AM and 101±3% for IB, and percent drug dissolution at 30 min was equal to 78± 6.5 % for AM and 101±3 % for IB. The method was successful and fast in evaluation the prepared tablets.

CONCLUSION

A simple validated method of analysis was developed to measure amoxicillin and ibuprofen in oro-dispersible tablets contains this combination. The method showed high sensitivity and accuracy according to USP and proved to be practical in the routine evaluation of the proposed combination tablets.

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