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ORIGINAL RESEARCH

Development and validation of a RP-HPLC method for quality control of oxantel pamoate, pyrantel pamoat and praziquantel in tablets

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ABSTRACT

In the present study, simple, rapid and precise HPLC methods were developed which would be useful for quality control of pharmaceutical dosage forms containing praziquantel (PRZ), pyrantel pamoate (PPA) and oxantel pamoate (OPA). The first method (M_1) was developed for the analysis of PRZ; separation was achieved using a reversed–phase column (4.6 x 150 mm, 5 µm) C18, a mobile phase comprising ACN:MeOH:20 mM phosphate buffer (0.2 % TEA, pH 4.5) (50:10:40, v/v/v) and UV detection at 210 nm. PPA and OPA were analysed simultaneously using a separate method (M_2) by employing the same column and flow rate. In accordance with the second method (M_2), detection wavelength was set at 295 nm and a mobile phase of ACN:MeOH:20 mM phosphate buffer (0.2 % TEA, pH 4.5) (12:3:85, v/v/v) was used. Benazepril hydrochloride (BZP) and paracetamol (PAR) were used as internal standards (IS) of the methods M_1 and M_2, respectively. Both methods were validated based on the parameters such as specifity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) besides system suitability tests. Forced degradation studies were performed to indicate specifity of the proposed methods. The methods were found linear over the concentration ranges of 0.5–7.5 µg/mL, 1–15 µg/mL and 2–40 µg/mL for PRZ, PPA and OPA, respectively. Correlation coefficients (r) of the regression equations were greater than 0.999 in all cases. The precision of the methods was demonstrated using intra- and inter-day assay RSD values which were less than 1% in all instances. Accuracy of the proposed methods was tested on placebo tablets spiked with known amounts of actives. Resulting recoveries of assays were in the range of 99.9–101.1 % whereas, those from commercial tablets were 99.4–100.8 %.

Keywords: Oxantel pamoate, Pyrantel pamoate, Praziquantel, HPLC, analytical method validation.

1. INTRODUCTION

Helmint infections which are influenced by parasites, affect more than one billion people in the world. Owing to the narrow spectrum of antihelmintic drugs it is needed to use combination chemotheraphy to control mixed infections (1). Combinations of oxfendazole-oxyclozanide (2) and fenbendazole-praziquantel-pyrantel pamoate (3) are the most common treatments of helmint infections. Another drug combination, which is the theme of our work, consists of oxantel pamoate, pyrantel pamoate and praziquantel and notably is being used to treat dogs (4). Although these three active ingredients are highly used in veterinary medicine, praziquantel and pyrantel pamoate exist in drug formulations for human.

To date, many HPLC methods have been developed to analyze praziquantel in various biological matrices (3, 5-9). HPLC analysis of pyrantel (10) alone or in combination with oxantel

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(11) and febantel-praziquantel (12) have also been reported.

Gonzalez *et al*. (5) determined praziquantel by using C18 column at 217 nm after solid phase extraction for preparing the sample. Enantiomers of praziquantel in human plasma were separated by Liu and Stewart by using cellulose-based chiral column and UV detector (6). Lerch and Blashcke (13) worked on praziquantel's metabolism in rat liver microsomes by the help of CE and LC-MS and revealed that *R-(-)* enantiomer metabolizes to *trans*- and *cis*-4-hydroxypraziquantel. As continuance, Meier and Blaschke (14) determined praziquantel's mono-, di-, trihydroxy metabolites and their glucuronide and sulphate conjugates in human urine by using CE-MS and LC-MS. The same research group identified *trans*- and *cis*-4-hydroxypraziquantel and an undefined monohydroxy metabolite in isolated rat hepatocytes by using gradient elution method (8). Schepmann and Blaschke (7), identified the new monohydroxy metabolite mentioned above, through combined MS^n and NMR techniques including a reversed phase HPLC column and ACN:H_2O (28:72 v/v) mobil phase, as 8-hydroxypraziquantel. Bonato *et al.* (15), reported the analysis of praziquantel and *trans*-4-hydroxypraziquantel in swine plasma samples by using a LC-MS-MS method which is comprised of a cyanopropyl column and MeOH:H_2O (3:7, v/v) plus 0.5% of acetic acid mobile phase.

A method for determination of praziquantel in human plasma was developed by the utilization of C18 reversed phase column and ACN:MeOH:H_2O (36:10:54 v/v/v) as mobile phase (9). Other report on HPLC determination of praziquantel in human plasma reveals the use of diazepam as internal standard using a similar reversed-phase column and ACN:H_2O (70:30, v/v), after liquid-liquid extraction of plasma samples (16). An enantioselective analysis method for praziquantel, (+)-(*S*)-praziquantel and (−)-(*R*)-4-hydroxypraziquantel enantiomers in human plasma by chiral LC-MS^2 method was reported. The method was reported to employ a Chiralpak AD column and hexane:isopropanol (75:25, v/v) mobile phase (17).

Praziquantel in bulk powder and its pharmacopoeial impurities were determined by using a calixarene column and mobile phase consisting of ACN and 25 mM ammonium acetate (18). The recent work describing determination of praziquantel in bulk and in synthetic mixtures through a reversed-phase high-performance liquid chromatography method was published in 2014 (19). Li *et al.* (20) reported HPLC and NMR – based analysis of praziquantel tablets to compare products from different manufacturers and to determine batch-to-batch variation from a single manufacture.

Through another work; praziquantel, fenbendazole and pyrantel pamoate combination was tried to be determined in dog plasma but not succeed by the reason of highly polar and basic character of pyrantel. Consequently, pyrantel was determined seperately due to its early retention time (3). For the determination of pyrantel pamoate in binary mixture with mebendazole C8 reversed phase column and phosphate buffer:ACN:TEA mobil phase were used at 290 nm (21). Determination of pyrantel tartarate in medicated feeds has been confirmed as a stable method of recent date (22). For the evaluation of pyrantel pamoate in binary mixture with oxantel pamoate, ACN with butylamine modifier mobile phase and C8 reversed phase column were used (11). Oxfendazole-oxyclozanide (2) binary mixture and combined preparations of similar antihelmintic drugs as mebendazole, fenbendazole, albendazole and their related impurities were determined together (23).

Nonetheless the absence of a report on determination of pyrantel pamoate, oxantel pamoate and praziquantel mixture from biological fluids or pharmaceutical dosage forms simultaneously, directs us to emphasize determination of these drugs. A specific, accurate, and precise method that could be applied to the quantitative analysis of tablets and other pharmaceutical preparations containing these three active ingredients was developed and validated for the determination of raw material and pharmaceutical product and for quality control, content uniformity and dissolution tests.

2. EXPERIMENTAL

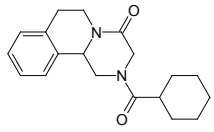
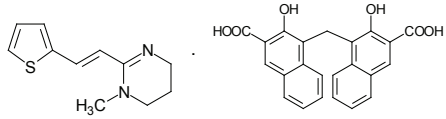
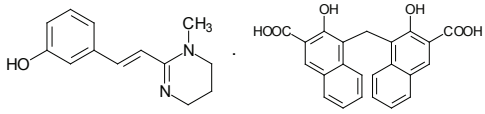
2.1. Materials and reagents

Pyrantel pamoate (PPA), oxantel pamoate (OPA), praziquantel (PRZ) standarts and pharmaceutical dosage forms (tablets) containing these APIs (Active Pharmaceutical Ingredients), were kindly provided by Topkim Topkapı Medicine Premix (İstanbul, Turkey). Benazepril HCl and paracetamol standarts were gifts from Novartis Pharmaceuticals (İstanbul, Turkey) and Drogsan (İstanbul, Turkey) respectively. Methanol and acetonitrile were of gradient grade and purchased from Merck company (Darmstadt, Germany). Triethylamine (TEA) and orthophosphoric acid (85%) were of analytical grade and procured from Fluka and Carlo-Erba Companies respectively. Potassium dihydrogen phosphate procured from Riedel-de Häen. Sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from Merck company (Darmstadt, Germany).

2.2. Instrumentation

The liquid chromatographic system used in the present study consisted of an Agilent Technologies 1100 series

Table 1. Structures and calculated physico-chemical properties of the APIs.

Structures and chemical names of the APIs	Formula & M.W. (g.mol^{-1})	Log P[1]	Log P[2]	pKa[1]
Praziquantel 2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino [2,1-*a*]isoquinolin-4-one 	$C_{19}H_{24}N_2O_2$ 312.406	2.42	2.74	-
Pyrantel pamoate *(E)*-1,4,5,6-Tetrahydro-1-methyl-2-[2-(2-thienyl)vinyl] pyrimidin, 4,4'-methylenebis [3-hydroxy-2-naphtoic acid] salt 	*Base* $C_{11}H_{14}N_2S$ 206.3083 Salt $C_{34}H_{30}N_2O_6S$ 594.678	2.69	2.47	11.00[a]
Oxantel pamoate *(E)*-1-Methyl-1,4,5,6-tetrahydro-2-[2-(3-hydroxyphenyl)vinyl] pyrimidine, 4,4'-methylenebis[3-hydroxy-2-naphtoic acid] salt 	*Base* $C_{13}H_{16}N_2O$ 216.279 *Salt* $C_{36}H_{32}N_2O_7$ 604.649	2.26	2.25	11.00 [a] 8.00 [b]

[1] Log P and pKa values were calculated using **ALOGPs** software (http://vcclab.org/lab/alogps/start.html).
[2] Log P values were calculated using **Molinspiration** software (http://www.molinspiration.com/).
[a] basic pK_a ; [b] acidic pK_a. All Log P and pK_a values of Pyrantel and Oxantel were calculated from their bases.

instrument equipped with a quaternary solvent delivery system and a model Agilent series G-13158 photodiode array detector. A Rheodyne syringe loading sample injector with a 50 µl sample loop was used for the injections of analytes. Chromatographic data were collected and processed using HP-Vectra VL-DOO DT software. The separation was performed at ambient temperature on a reversed phase Waters Symmetry C18 Column (150 mm x 4.6 mm; 5µm particle size). A Waters Symmetry C18 analytical guard column packed with the same sorbent was used.

SMILES were generated from the structures using the ACD/ ChemSketch version 8.0 molecular editor (http://www.acdlabs.com) and then log P and pK_a values were calculated using ALOGPS 2.102 logP/logS calculation software (24, 25). Validity of the Log P values were also checked using another online software, Molinspiration online property calculation toolkit (26). The calculated log P and pK_a values for all the compounds are given in Table 1.

2.3. Mobile phases

Two kinds of mobile phase systems were used in our work :

M_1 : ACN:MeOH:20 mM phosphate buffer (0.2 % TEA, pH 4.5) (50:10:40, v/v/v)

M_2 : ACN:MeOH:20 mM phosphate buffer (0.2 % TEA, pH 4.5) (12:3:85, v/v/v)

Preparation of phosphate buffer : 2.722 g KH_2PO_4 was dissolved in sufficient bidistilled water and 2 ml TEA was added to produce 1000 ml. The final pH of the solution was adjusted to the 4.5 with orthophosphoric acid.

2.4. Standard stock solutions

Stock solutions of OPA, PPA, PRZ, PAR and BZP : 20 mg of each was weighed and dissolved in methanol to produce 100 ml by ultrasonication for 10 minutes. The volumetric flasks were wrapped with foil paper to keep out of light and preserved at +4°C.

2.5. Standard stock solutions for precision studies

The precision of the proposed method was assessed as repeatability performing five replicate injections of three different sample solutions with concentations 0.6-2.0-6.0 µg/ml for PRZ, 12.5 µg/ml for PPA and 3.0-15.0-37.5 µg/ml for OPA

2.6. Sample preparations

Ten tablets were weighed, their mean weight were determined as 1050.03 mg and then they were finely powdered. 38.25 mg of powder was transferred into a 100

ml volumetric flask and diluted to 100 ml with methanol, sonicated for 20 min and 10 ml sample taken from this solution was centrifuged at 3000 rpm for 15 min. To a 1 ml aliquot from supernatant was added 100 µl of benazepril HCl stock solution and 750 µl of paracetamol stock solution the made up the volume of 10 ml with mobile phase.

2.7. Chromatographic conditions

Two methods used for chromatographic analysis of the APIs can be described as follows.

M_1 : A reversed-phase Waters Symmetry C18 column with a particle size of 5µm and dimentions of 4.6 x 150 mm were used. The mobile phase consisted ACN:MeOH:20 mM phosphate buffer (0.2% TEA, pH 4.5) (50:10:40, v/v/v) and delivered at a flow rate of 1.5 ml/min. Injection volume was 50 µl. The analytes were monitored using a PDA detector at 210 nm (bandwidth: 4 nm). The buffer contained 20 mM KH_2PO_4 and 0.2% TEA; and the final pH were adjusted to pH 4.5 with H_3PO_4.

M_2 : A reversed-phase Waters Symmetry C18 column with a particle size of 5 µm and dimentions of 4.6 x 150 mm were used. The mobile phase consisted of ACN:MeOH:20 mM phosphate buffer (0.2 % TEA, pH 4.5) (12:3:85, v/v/v) and delivered at a flow rate of 1.5 ml/min. Injection volume was 50 µl. The analytes were monitored using a PDA detector at 295 nm (bandwidth: 4 nm).

3. RESULT and DISCUSSION

3.1. Determination of the mobile phase

The pharmaceutical dosage form which is the theme of our work consists of 50 mg of praziquantel (PRZ), 140 mg of pyrantel pamoate (PPA) and 545 mg of oxantel pamoate (OPA) for each tablet. These three active ingredients are being governed in veterinary medicine together with human use. We planned this work on the basis of absence of an analytical method for the determination of pharmaceutical dosage forms containing PRZ, PPA and OPA combinations by HPLC. For accessing the optimum chromatographic conditions lots of attemps were held. The first attempt was trying to use water included mobile phases. By using mobile phase systems which consist of ACN:MeOH:water at different levels, PRZ's peaks were evaluated at acceptable retention time but because of the basic characters of PPA and OPA high tailing factor, unacceptable retention profile and peaks holding trogether with the void volume occured. For later-dated experiences, buffer solutions of KH_2PO_4 and orthophosphoric acid (for pH adjustment) were prepared. When the pH of the buffer solution was acidic the wide peak shapes of pyrantel and oxantel got narrower but acceptable elution and retention time were not assessed. Changing pH did not affect non-basic PRZ; peak shape and retention time of it didn't vary. When PPA and OPA were well-resolved from each other there was no eluting potential for PRZ and very late eluting potential for pamoic acid that could interfere the following injections. As PRZ had an acceptable retention time, PPA and OPA were not well-resolved from each other and from the void volume therefore an ion-pair forming agent, sodium hexane sulfonate (PIC B-6), was used. During this trial, either no well-resolving profile for PPA and OPA or late retention time for PRZ were carried out. Consequently gradient elution method was tried to determinate the multi-component tablet. By the help of gradient elution PRZ, PPA and OPA were eluted with success but some disadvantages of the system were confirmed. If we considered the ratio of active ingredients of tablet there would be no sufficient resolving profile for PPA and OPA. It was necessary to detect PRZ at 210 nm but shift and noise were occured at this wavelenght. Time of one analysis lasted at least 30 mins. Tailing of pamoic acid peak could interefere with PRZ. After all these attempts a method to analyse the four component of the tablet can not be optimized so two different methods were enhanced for PPA and OPA together and PRZ alone. In 1998, Morovján *et al*. (3) determined praziquantel, fenbendazole and pyrantel pamoate in dog plasma by facing the same problems so that they determined pyrantel pamoate alone and praziquantel–fenbendazole together. By using 20 mM KH_2PO_4 buffer and orthophosphoric acid for pH adjustment to 4.5, the best results were obtained. TEA was added to the mobile phase for getting reproducibility and avoiding secondary interaction between silanol groups and PPA, OPA as the cause of unacceptable peak shapes (27). Since PRZ has been nonbasic it was not affected by this change. After testing the combinations of ACN-MeOH-20 mM KH_2PO_4 (0.2% TEA, pH 4.5) with ratios 10:50:40, 30:30:40, 40:20:40 and 50:10:40 v/v/v it was approved to work with the fourth one to determine PRZ. This method was rapid, it lasted nearly 5 mins and pyrantel, oxantel and pamoic acid were eluted at void volume so no interference with PRZ and other injections. Flow rate was 1.5 ml/min and wavelength for detection was 210 nm for this method (M1). For acceptable retention times appertaining PPA and OPA, ratio of organic solvent was reduced in M1 system and a new system (M2) of ACN:MeOH:20 mM KH_2PO_4 (0.2% TEA, pH 4.5) 12:3:85 v/v/v was developed. Flow rate remained the same at M2 system but wavelength for detection was set to 295 nm to avoid noise and to achieve higher absorption of PPA and OPA. Required time for one analysis by employing M2 system was shorter than 6 mins and there

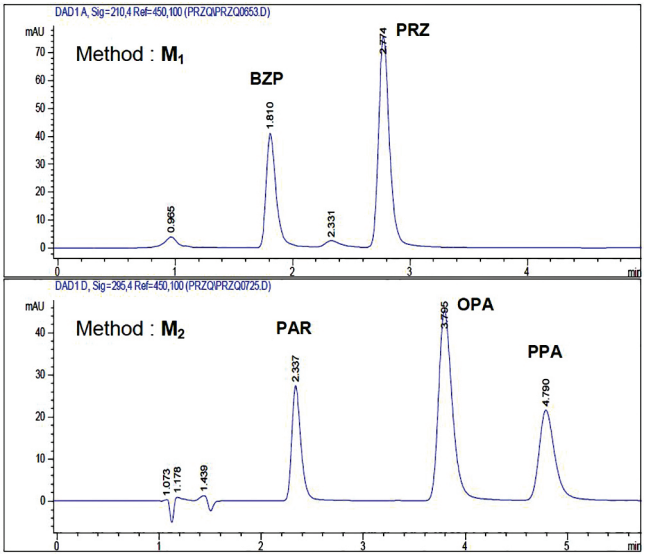


Figure 1. Typical chromatograms obtained from standard solutions of OPA, PPA and PRZ (Sample concentrations: PRZ = 3 µg/mL; BZP = 2 µg/mL; PPA = 5 µg/mL; OPA = 10 µg/mL; PAR = 15 µg/mL).

were no peak accounting for neither PRZ nor pamoic acid. Internal standarts; BZP for M1 system and PAR for M2 system were used to eliminate errors of manual injection.

3.2. Validation of the analytical method

The aim of validation of an analytical method is to state whether it serves the aim or not (28). A developed method should pass the tests: precision, accuracy, limit of detection, limit of quantitation, specificity, linearity and range, ruggedness, robustness. Specificity symbolizes the selectivity of the system and means that a peak represents only one substance and no co-elution with the other ingredients of tablet, internal standards, impurities and degradation products. In our work, PRZ, PPA and OPA were discriminated from each other and internal standards on the basis of qualitative and quantitative aspect with acceptable resolution values (Please see Figure 1). By the help of recovery process it was confirmed that inactive ingredients of tablet did not affect the analysis.

Since reference standards for impurities of active ingredients have not been supplied accelerated degradation studies were performed to provide an evidence for the specificity of the proposed method. Degradation experiments were designed using acid, base, oxidative agents, heat, UV and direct daylight and it was shown that degradation products' peaks were quantitatively well-resolved from the peaks of active ingredients. Peak homogeneity of the compounds was checked using an Agilent 1100 Diode array detector (DAD) and it was demonstrated that a peak represents an active ingredient. Losses due to degradation experiments that drew

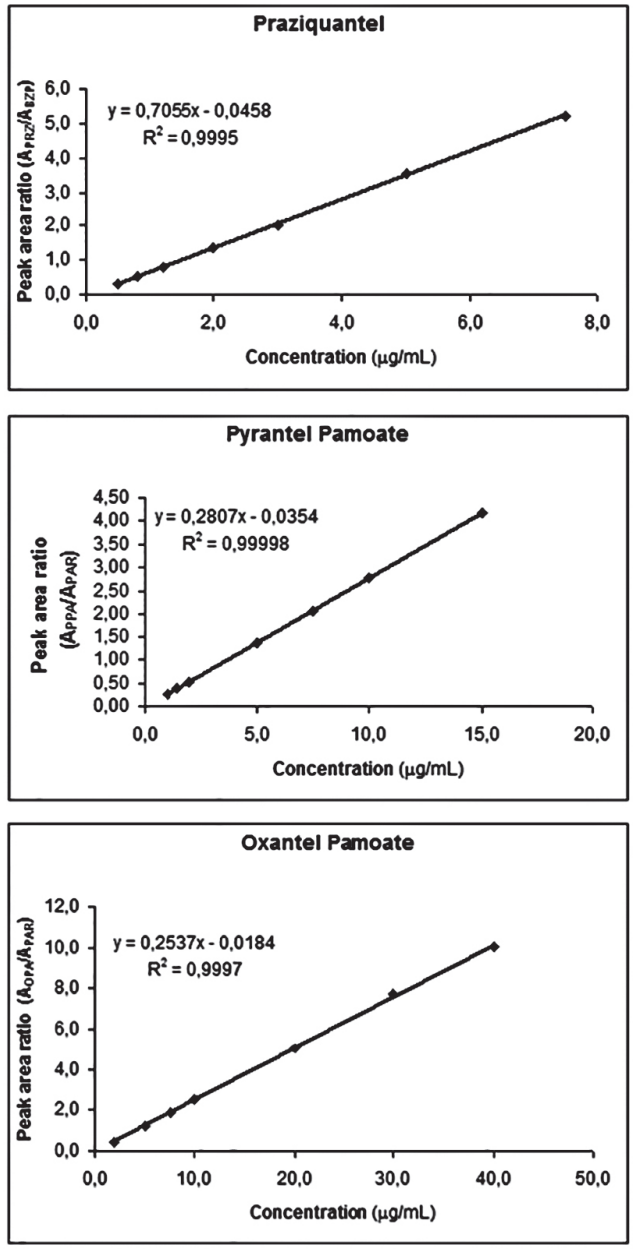


Figure 2. Calibration plots of OPA, PPA and PRZ.

Table 2. Characteristics of **PSE** and **CET** calibration plots.

	PRZ	PPA	OPA
Linearity range (µg.mL^{-1})	0.5–7.5	1–15	2–40
Slope*	0.7055	0.2807	0.2537
Intercept*	– 0.0458	– 0.0354	– 0.0184
Standard error of the slope	0.00066	0.00056	0.00028
Standard error of the intercept	0.00134	0.00091	0.00056
Correlation coefficient (r)	0.9997	0.9999	0.9998
Limit of detection (µg.mL^{-1})	0.014	0.024	0.016
Limit of quantification (µg.mL^{-1})	0.043	0.072	0.050

* : Mean of five injections.

Table 3. Summary of intra-day (repeatability) and inter-day (intermediate precision) variability data for the analysis of PRZ, PPA and OPA.

	C_{added} (µg/mL)	Intra-day			Inter-day		
		Found* (µg/mL)	% Recovery	RSD	Found* (µg/mL)	% Recovery	RSD
PRZ	0.6	0.60	100.4	0.25	0.60	99.3	0.80
	2.0	1.98	99.2	0.29	1.99	99.4	0.33
	6.0	5.97	99.6	0.18	5.97	99.5	0.44
PPA	1.2	1.21	100.4	0.42	1.20	99.8	0.12
	5.0	4.98	99.5	0.62	5.02	100.4	0.18
	12.5	12.44	99.5	0.23	12.44	99.5	0.49
OPA	3.0	2.99	99.7	0.32	3.03	100.9	0.40
	15.0	15.04	100.2	0.27	14.94	99.6	0.30
	37.5	37.64	100.4	0.27	37.80	100.8	0.13

*: Mean of five injections.

Table 4. Resolution of PRZ, PPA and OPA in laboratory-made mixtures using the proposed method.

Added (µg/mL)			**Found** (µg/mL)			**% Recovery**		
PRZ	**PPA**	**OPA**	**PRZ**	**PPA**	**OPA**	**PRZ**	**PPA**	**OPA**
2	5	5	2.00	4.98	5.07	100.2	99.6	101.4
2	5	10	2.00	4.97	9.97	99.9	99.3	99.7
2	5	15	1.99	4.93	15.20	99.4	98.7	101.3
2	5	20	2.00	4.94	20.26	99.8	98.7	101.3
2	5	30	2.01	5.01	30.39	100.3	100.2	101.3
					mean	**99.9**	**99.3**	**101.0**
					% RSD	**0.35**	**0.67**	**0.71**
2	1.5	15	2.01	1.48	15.08	100.5	98.9	100.5
2	2	15	1.99	1.98	15.06	99.6	99.1	100.4
2	5	15	2.01	4.96	15.08	100.3	99.1	100.6
2	8	15	2.00	7.92	14.91	100.1	99.0	99.4
2	12	15	1.99	11.91	14.99	99.3	99.3	99.9
					mean	**99.9**	**99.1**	**100.2**
					% RSD	**0.51**	**0.13**	**0.51**
0.6	5	15	0.61	4.99	15.10	101.3	99.8	100.7
1	5	15	1.01	5.03	15.10	101.2	100.6	100.6
2	5	15	2.03	5.04	15.07	101.3	100.9	100.5
4	5	15	4.01	5.05	15.13	100.2	101.0	100.8
6	5	15	6.08	5.02	15.09	101.4	100.3	100.6
					mean	**101.1**	**100.5**	**100.6**
					% RSD	**0.50**	**0.48**	**0.13**

attention were caused by heating PRZ with acid and PRZ, OPA with base. It was observed that, direct exposure to daylight and UV irradiation decompose pyrantel whereas other two APIs did not. At the end of other degradation reactions no notable degradation product were detected.

Table 5. Stathistical analysis of assay results and recovery experiments in the placebo tablets and commercial samples.

	Analyst – I			Analyst – II		
	PRZ	**PPA**	OPA	PRZ	**PPA**	OPA
Label claim (mg)	50	140	545	50	140	545
Mean of amount found (mg)*	50.413	139.721	542.906	49.922	139.153	544.715
Confidence limits	± 0.330	± 0.719	± 3.278	± 0.462	± 0.534	± 3.556
Recovery %	100.826	99.800	99.616	99.843	99.395	99.948
RSD %	0.624	0.491	0.576	0.882	0.242	0.622
t – test**	2.225	1.819	0.961	$t_{theoretical}$ = 3.17 ***		
Placebo tablets – added amount (mg)	54.781	153.388	597.117	53.825	150.710	586.694
Mean of amount found (mg)	55.054	154.292	600.683	54.424	150.608	592.972
Confidence limits	± 0.212	± 0.854	± 1.525	± 0.430	± 1.235	± 2.119
Recovery %	100.497	100.589	100.597	101.112	99.932	101.070
RSD %	0.367	0.527	0.242	0.753	0.782	0.341
t – test**	1.780	1.705	2.748	$t_{theoretical}$ = 3.17 ***		

* Mean values represent six determinations.
** *t*-tests were calculated using mean recoveries.
*** $t_{theoretical}$ value was taken from *t*-table for 99% confidence level and N=12.

In the wake of calibration experiments, concentration levels ranging from 0.5-7.5 µg/ml for PRZ, 1-15 µg/ml for PPA and 2-40 µg/ml for OPA were found in linear correlation with detector response. Correlation coefficients of regression equations were calculated as 0.9995 for PRZ, 0.99998 for PPA, 0.9996 for OPA due to high linearity (Please see Figure 2 and Table 2). By using calibration curves and LOD=3.3.σ/S and LOQ=10.σ/S criterions limit of detection (LOD) for PRZ, PPA and OPA were calculated as 0.014 µg/ml, 0.024 µg/ml and 0.016 µg/ml the limit of quantitation (LOQ) for PRZ, PPA and OPA were calculated as 0.043 µg/ml, 0.072 µg/ml, 0.050 µg/ml respectively.

The precision of the proposed method was assessed as repeatability and intermediate precision. Recovery from placebo tablets and designation of the effect of different analyst on quantitative determination of compounds were planned as another intermediate precision experiment. The required results are depicted in Table 3.

According to the data given in Table 4, changes between the concentration ratios have no negative effect on repeatability. Proximity between the data required by prescribed method and the actual data can be defined as

Table 6. Recovery of APIs after accelerated degradation experiments under several stress conditions.

Stress conditions*	Time	Recovery (%)			Relative retention times (RRT) of degradation products**		
	(h)	PRZ	OPA	PPA	PRZ	OPA	PPA
Fresh standard (control)	0	100	100	100	0.846, 0.714	-	0.698
Mobile phase, stored in dark at room temperature	24	99.573	98.98	99.306	0.846, 0.714	-	0.699
Exposed to direct sun light in MeOH at room temperature	24	98.808	98.864	**42.949**	0.844, 0.714	0.698	0.694
Exposed to UV irradiation in MeOH at room temperature UV (254 nm)	10	97.224	97.798	**78.057**	0.845, 0.715	0.689	0.238, 0.696
MeOH, heating at 80 ºC	4	97.64	98.224	95.104	0.844, 0.714	-	0.697
0.5 N HCl, heating at 80 ºC	4	**89.334**	98.231	94.439	0.330, 0.437	-	0.700
					0.714, 0.846		
0.5 N NaOH, heating at 80 ºC	4	**3.266**	**4.613**	97.256	0.333, 0.418	0.748, 0.805	0.694, 0.763
					0.845		1.339
%3 H_20_2, heating at 80 ºC	2	95.358	98.28	97.178	0.714, 0.846	-	0.696

accuracy and this work was held on placebo tablets having similar concentration with the test concentration. Two analyst worked on this attempt and the results were evaluated with % recovery. Upon getting high recovery values with placebo tablets, the developed method was decided to be applied to pharmaceutical dosage forms. Mean values of assay results obtained from two different analysts were analyzed through *t*-test if there's a statistically significant difference. Results showed that *t* values for placebo tablets and pharmaceutical products are not more than 3.17 which is the theoretical value for N=12 at 99% confidence limit, indicating no significant difference between the mean contents of the APIs obtained by two different analysts. The data gained by recovery studies were summarized in Table 5 and Table 6.

In accordance with USP 23, system suitability tests are an integral part of a liquid chromatographic method, and they were used to verify that the proposed method was able to produce good resolution between the peaks of interest with high reproducibility (29). The system suitability was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their peak area, theoretical plates (*N*), resolution (*R*) and tailing factors (*T*). System suitability requirements for OPA, PPA and PRZ were a R.S.D. of peak areas and retention times less than 1%, peak resolution (*R*) greater than 2.0 between two adjacent peaks for three analytes, theoretical plate numbers (*N*) at least 2000 for each peaks and USP tailing factors (*T*) less than 1.5. The results of system suitability test in comparison with the required limits can be shown in Table 7. Calculated k′ values of PRZ, PPA and OPA are 1.629, 3.269 and 2.381 respectively reveals well-resolution and no need to prolong the time of analysis. According to the results presented, the proposed method fulfils these requirements within the accepted limits (Please see Figure 3).

Table 7. System suitability results of the developed method.

Method	Compound	*k'*	α	R	N	T	RSD
M_1	BZP	0.716	-	-	2462	1.370	0.32
	PRZ	1.629	2.275	6.298	4848	1.238	0.43
M_2	PAR	1.079	-	-	2860	1.326	0.29
	OPA	2.381	2.206	6.010	2380	1.242	0.21
	PPA	3.269	1.373	3.068	3225	1.245	0.19

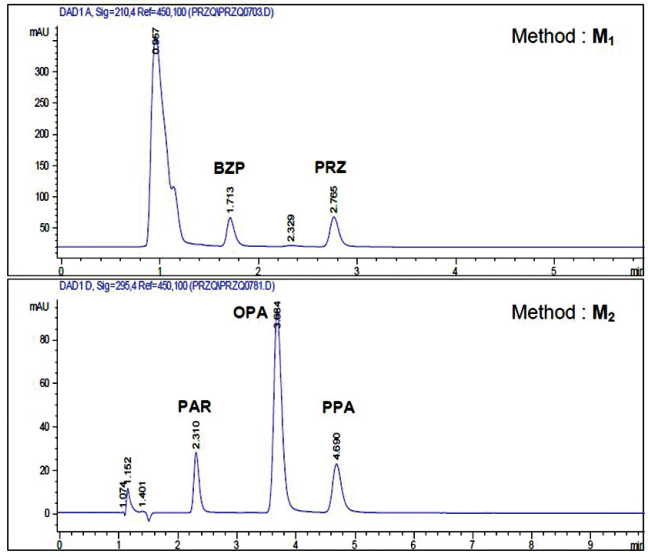


Figure 3. Typical chromatograms obtained from the analysis of tablet formulation comprises of OPA, PPA and PRZ.

4. CONCLUSION

According to the results the proposed method was found to be specific, accurate, precise and fast. There is no need for ion-pairing agents and gradient elution and also no need for

liquid-liquid and solid phase extraction to prepare samples. The time of analysis lasts for 5 and 6 minutes for the systems M1 and M2, respectively. Considering the advantages of the method; it can be used for quality control, content uniformity and stability tests of PRZ, PPA and OPA.

Oksantel pamoat, pirantel pamoat ve prazikuantel içeren tabletlerin kalite kontrolü için bir zıt faz HPLC yönteminin geliştirilmesi ve validasyonu

ÖZET

Bu çalışma kapsamında, prazikuantel (PRZ), pirantel pamoat (PPA) ve oksantel pamoat (OPA) içeren farmasötik dozaj şekillerinin kalite kontrolünde kullanılabilecek basit, hızlı ve duyarlı yüksek basınçlı sıvı kromatografisi (HPLC) yöntemleri geliştirilmiştir. PRZ analizleri için geliştirilen birinci yöntemde (M_1) Waters Symmetry C18 (4.6 x 150 mm, 5 µm) zıt faz kolonu ile birlikte hareketli faz olarak 1.5 mL/dk akış hızında ACN – MeOH – 20 mM fosfat tamponu (% 0.2 TEA, pH 4.5) (50:10:40, h/h/h) kullanılmış ve DAD dalga boyu 210 nm'de çalışılmıştır. PPA ve OPA'nın bir arada analizi için geliştirilen diğer yöntemde (M_2) ise aynı kolonda ve akış hızında çalışılırken, detektör dalga boyu 295 nm olarak belirlenmiştir. M_2 yönteminin hareketli fazının içeriği ACN – MeOH – 20 mM fosfat tamponu (% 0.2 TEA, pH 4.5) (12:3:85, h/h/h) şeklinde belirlenmiştir. M_1 yöntemiyle analizi yapılan PRZ için benazepril hidroklorür (BZP), M_2 yöntemiyle gerçekleştirilen PPA ve OPA analizlerinde ise parasetamol (PAR) iç standart olarak kullanılmıştır. Geliştirilen yöntemlerin validasyonunda, özgünlük, doğrusallık, kesinlik, doğruluk, tayin alt sınırı ve miktar tayini alt sınırı gibi parametreler belirlenmiş; sistem uygunluk testleri yapılmış; ayrıca, planlanan hızlandırılmış bozundurma deneyleriyle yöntemin özgünlüğü gösterilmiştir. Yapılan doğrusallık çalışmalarında PRZ, PPA ve OPA için sırasıyla 0.5-7.5 µg/ml, 1-15 µg/ml ve 2-40 µg/ml aralıklarında elde edilen regresyon eşitliklerinin korelasyon katsayıları 0.999'dan yüksek bulunmuş ve yöntemlerin doğrusallığı gösterilmiştir. Yöntemlerin kesinliğini gösteren gün içi ve günler arası tekrarlanabilirlik deneylerinde elde edilen bağıl standart sapma (BSS) değerleri daima % 1'den küçük bulunmuştur. Doğruluk deneylerinde; geliştirilen yöntemlerin plasebo tabletlere uygulanmasından elde edilen geri kazanım değerleri % 99.9–101.1 aralığında bulunmuştur. Bu değerlerin, bitmiş ürünlerde % 99.4–100.8 aralığında değiştiği saptanmıştır.

Anahtar sözcükler: Oksantel pamoat, Pirantel pamoat, Prazikuantel, HPLC, analitik yöntem validasyonu.

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