Asian Journal of Chemistry; Vol. 34, No. 10 (2022), 2561-2566

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23853

Development and Validation of Stability Indicating Mass Compatible RP-HPLC Method for Simultaneous Estimation of Assay and Related Substances in Methylprednisolone Acetate Injectable Suspension

C. BALAKRISHNAN[1], K.R.S. PRASAD[1,*] and K. SURESH BABU[2]

[1]Department of Chemistry, Koneru Lakshmaiah Educational Foundation, Vaddeswaram-522302, India
[2]Department of Chemistry, Marri Laxman Reddy Institute of Technology and Management, Hyderabad-500043, India

*Corresponding author: E-mail: krsprasad_fed@kluniversity.in

Received: 27 March 2022; *Accepted*: 28 April 2022; *Published online*: 19 September 2022; AJC-20956

A new stability indicating mass compatible RP-HPLC method was developed and validated for the simultaneous estimation of assay and related substances of methylprednisolone acetate (MPA) in methylprednisolone acetate injectable suspension. Chromatography was carried out with C_{18} column (100 mm × 4.6 mm, 3.5 µ particle size) with an isocratic elution of mobile phase composed of 1 g/L Ammonium acetate and Acetonitrile in the ratio of 67:33 v/v at a flow rate of 1.5 mL/min. The column oven temperature was maintained at 50 ºC and the detection was carried out using UV detector at 254 nm. Validation parameters such as system suitability, specificity (matrix interference and forced degradation), linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), solution stability and robustness were performed according to ICH guidelines. The retention time for MPA was about 7 min. Methylprednisolone acetate (MPA) and its impurities were well separated from each other. The percentage mean accuracy of MPA was found to be 99.1% for assay and ranged from 101.4% to 104.8% for known impurities. The % relative standard deviation (RSD) for the six replicate assay results was found less than 1%. The method is linear in the range from 0.2 µg/mL to 600 µg/mL (*i.e.* 0.05% to 150% of the test concentration). The correlation coefficient for linearity was found to be greater than 0.999 for MPA and its impurities. Limit of detection and limit of quantification was demonstrated to be 0.017%w/w and 0.05%w/w, respectively. The validated method is simple, mass compatible, fast, specific, stability indicating, accurate, linear, precise, rugged, sensitive and robust.

Keywords: Methylprednisolone acetate, Stability indicating assay, RP-HPLC.

INTRODUCTION

Methylprednisolone acetate (MPA) is a synthetic corticosteroid used to treat inflammation [1]. Methylprednisolone is a prednisolone derivative glucocorticoid with higher potency than prednisone [2]. Treatment regimen consisting of reduced immunosuppressant use and low dose methylprednisolone-based therapy for the COVID-19 pneumonia is successful [3]. Methylprednisolone acetate injectable suspension DEPO-MEDROL® is approved by USFDA for an anti-inflammatory glucocorticoid for intramuscular, intra-articular injection. It is available in two strengths (40 mg/mL, 80 mg/mL) [4].

USP monograph of MPA injectable suspension [5] has a HPLC based assay method that involves use of L3 stationary phase and sample preparation by liquid-liquid extraction using chloroform as extraction solvent. Quantification is by internal standard method and the extraction procedure is complex and time-consuming. Related substances method is not part of USP monograph of MPA injectable suspension as well as other available USP monographs like neomycin and MPA cream [6]. The USP monograph of MPA drug substance [7] consists of related substances method but no known impurities are listed. The European Pharmacopoeia (EP) [8] monograph contains two separate methods for assay and related substances of MPA drug substance and does not have method for drug product. Tsuji & Binns [9] demonstrated the suitability and superiority of micro bore column with various pharmaceutical compounds including MPA, however this study shows only separation of MPA and internal standard (prednisone) but not its impurities. Similarly, Signoreiti *et al.* [10] developed four different HPLC

methods (2 RPLC and 2 NPLC) and concluded that one of the RPLC method (RP1) showed higher resolution capability than other three methods. However, the runtime of RP1 method is very long (120 min), sample preparation involves evaporation to dryness and then dissolved to get a required concentration of MPA and the method for analysis of drug substance and suitability of the method for preservative formulation was not demonstrated.

There are few HPLC [11-13] and LCMS [14] methods reported for estimation of MPA in human plasma, synovial fluid and rat plasma. HPLC method [15] available for simultaneous analysis of prednisone, prednisolone and its major hydroxylated metabolites in urine. Solomun *et al.* [16] followed USP method for assay and developed new related substance method for methylprednisolone hydrogen succinate and methylprednisolone sodium succinate where the run time is 70 min. There are other few HPLC methods [17,18] for determination of methylprednisolone sodium succinate and its related substances from methylprednisolone sodium succinate for injection. The above discussed methods are mainly suitable for estimation of MPA peak at lower level in plasma and other salt forms of methylprednisolone and would not be suitable for estimation of assay in presence of related substances, excipients and preservatives in pharmaceutical products.

The literature survey indicates a paucity of short and simple stability indicating RPLC method for the simultaneous estimation of assay and related substances of methylprednisolone acetate (MPA) in methylprednisolone acetate injectable suspension. Hence, an attempt has been made to develop and validate a short, simple, stability indicating mass compatible RPLC method for the simultaneous estimation of assay and related substance of MPA in injectable suspension pharmaceutical product according to ICH guidelines for validation of analytical procedures [19].

EXPERIMENTAL

HPLC grade ammonium acetate, HPLC grade acetonitrile and all other chemicals were obtained from Merck chemical division, India. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Methylprednisolone acetate reference standard was procured from USP (United states pharmacopoeia). Impurities (prednisolone (impurity-1), prednisolone acetate (impurity-2) and methyl prednisolone (impurity-3)) were procured from contract research laboratories.

Instrument and chromatographic conditions: HPLC equipped with DAD was employed in this method. Empower software was used for data acquisition and processing. Waters Sunfire® C_{18} column (USP L7) (100 mm length, 4.6 mm internal diameter and 3.5 µ particle size) with an isocratic mobile phase composed of 1 g/L ammonium acetate and acetonitrile in the ratio of 67:33 v/v at a flow rate of 1.5 mL/min. The column oven temperature was maintained at 50 ºC and the detection was carried out using UV detector at 254 nm. Standard and samples were dissolved in diluent (Buffer: acetonitrile in the ratio of 25:75 v/v) to get a concentration of 0.4 mg/mL MPA. 10 µL of standard solution was injected in 5 replicates followed by duplicate injections of sample solutions with a runtime of 15 min for standard and 20 min for sample. The % RSD (Not more than 2.0%) for the peak area of MPA from five replicate standard injections, theoretical plates (not less than 4000) and tailing factor (not more than 1.5) was monitored as part of system suitability.

Analytical method development

Challenges in existing assay method of drug product: The USP monograph method for assay is based on liquid-liquid extraction with chloroform as extraction solvent. The quantification is internal standard method and height ratio of MPA and internal standard peaks are used for calculating the assay. The height of the peak is highly dependent on column performance. The extraction procedure is complex, time consuming and expensive. Higher level of variation is observed for the assay due to the complexity of the method. Hence, an attempt has been made to develop a simple and cost-effective method for the simultaneous estimation of assay and related substances by RPLC. The stability indicating RPLC method was developed by varying different chromatographic conditions including buffer, mobile phase ratio, solvents, columns, flow rate, column oven temperature using established HPLC method development strategies [20-22].

Selection of buffer for mobile phase: Ammonium acetate was chosen as buffer due to its mass compatibility and pK_a being away from the pK_a of MPA.

Selection of acetonitrile as organic phase for mobile phase: Acetonitrile has a low UV cut-off and exhibited selectivity for the impurities and MPA.

Screening of diluent: The initial diluent selected was mixture of 1 N HCl, 0.5 N KCl, 0.5 M sodium acetate and water in the ratio of 20:150:50:780 based on EP monograph of MPA drug substance. The solubility of MPA suspension found to be less in this diluent and more than 1000 mL of diluent was needed to dissolve 1 vial of 80 mg/mL drug product (400 mg/5 mL). Further, solubility was checked in different composition of ammonium acetate buffer and acetonitrile to increase the solubility to minimize diluent quantity required for sample preparation. MPA was found soluble at a concentration of about 4 mg/mL in the diluent with composition of ammonium acetate buffer and acetonitrile in the ratio of 25:75 v/v, respectively. This significantly reduces the diluent volume required to completely dissolve 1 vial of drug product. The diluent of ammonium acetate buffer and acetonitrile in the ratio of 25:75 v/v is finalized.

Optimization of sample preparation: MPA drug product is suspension and tends to settle over a period of time, so it is important to homogenize the vial prior to sampling and also select suitable volume of suspension for dilution. Several homogenization techniques like shaking, stirring, cyclomixer were tried and use of cyclomixer was finalized.

Standard and sample preparation: Standard and sample were sonicated in diluent and suitably diluted to get a concentration of 0.4 mg/mL of MPA in final solution.

Analytical method validation and results: The analytical method validation for the simultaneous estimation of assay and related substance of methylprednisolone acetate (MPA)

in methylprednisolone acetate injectable suspension was conducted in accordance with ICH guidelines [19]. Analytical method validation parameters such as system suitability, specificity (matrix interference and forced degradation), linearity, precision (method precision and intermediate precision), accuracy, limit of detection (LOD), limit of quantitation (LOQ), solution stability and robustness were performed according to ICH guidelines to demonstrate the suitability of the method for the estimation of assay and related substances.

System suitability: System suitability was demonstrated by evaluating % RSD (not more than 2.0%) for the peak area of MPA from five replicate standard injections, theoretical plates (not less than 4000) and tailing factor (not more than 1.5). The %RSD of peak area of MPA from 5 replicate injection was found to be 0.2%, theoretical plates are 7398 and tailing factor was 1.0.

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present *e.g.*, degradation products, related substances and excipients [19]. The specificity of the analytical method was evaluated by injecting blank, placebo, individual known impurities and sample spiked with known impurities at 0.5% level. Overlaid chromatograms of blank, placebo, sample and spiked sample is presented in Fig. 1.

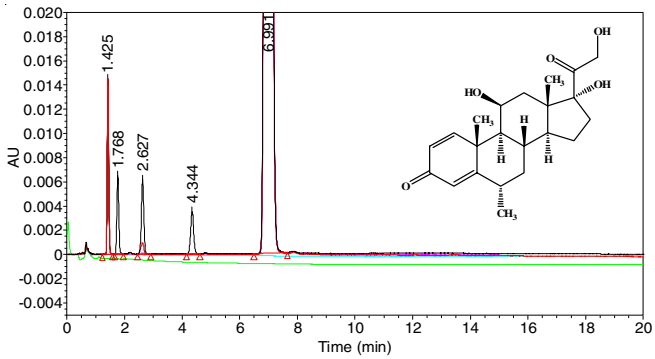

Fig. 1. Overlaid chromatograms of blank, placebo, standard, sample, spiked sample (RT 1.425 min-benzyl alcohol, RT 1.768 min-impurity-1, RT 2.627 min-impurity-3, RT 4.344 min-impurity-2, RT 6.991-MPA)

Forced degradation studies: Drug product was treated with acid, base, hydrogen peroxide and also exposed to thermal and photolytic condition in order to induce deliberate degradation of drug product. Degraded samples were injected into a HPLC equipped with DAD detector. Stability indicating nature of the method was proved by assessing the peak purity data of MPA in stressed sample.

Method precision: Precision (repeatability) was demonstrated by analyzing six replicate preparations of test solutions. The %RSD for assay, related substances of six-replicate sample preparations are calculated.

Intermediate precision/ruggedness/reproducibility: Intermediate precision was demonstrated by analyzing six replicate preparations of test solutions using different HPLC and different column on different days. The % RSD for assay and related substances of 12 replicate (n = 6 for method precision and n = 6 for intermediate precision) are calculated.

Accuracy: Accuracy is closeness of value with true value or an accepted reference value [19]. The accuracy of the analytical method for assay was demonstrated for MPA at 50%, 100% and 150% of nominal test concentration in triplicate. The accuracy of all the known impurities was demonstrated from LOQ to 200% considering 0.5% as nominal level of impurities. The % mean recovery and % relative standard deviation was calculated at each level.

Linearity: The linearity of the method is its ability to show that the absorbance is directly proportional to the concentration of analyte in the sample [19].

Linearity of the analytical method was demonstrated by plotting peak area and concentration of MPA in the range of 50% to 150% of the nominal test concentration for assay and established the regression line with method of best fit. Correlation coefficient, regression coefficient, slope and intercept of the regression line were calculated. Additionally, linearity of MPA was demonstrated from LOQ (0.05% of test concentration) to 120% of test concentration to show that the response of MPA is linear from LOQ to 120% of test concentration and suitable for the determination of related substances. The linearity for impurities is demonstrated from LOQ to 200% of nominal limit.

Limit of detection (LOD) and limit of quantitation (LOQ): Methylprednisolone acetate (MPA) and its impurities were prepared at a concentration equal to the reporting threshold of 0.05% of test concentration and demonstrated LOQ. LOD was demonstrated by preparing 1/3rd concentration of LOQ *i.e.* 0.017%. Signal to noise ratio for MPA and its impurities are calculated.

Range: The range of an analytical procedure is the interval between the lower and upper concentration of analyte with demonstrated precision, accuracy and linearity [19]. The range of the method is 50% to 150% of test concentration for assay and LOQ to 200% of 0.5% limit for impurities.

Robustness: Robustness of the analytical method was investigated under a variety of conditions by deliberate change in composition of buffer and acetonitrile in the mobile phase (buffer, acetonitrile in the ratio of 67:33 (control), 65:35 (organic plus) and 69:31 (organic minus)), flow rate (1.5 mL/min (control), 1.3 mL/mL (flow minus) and 1.7 mL/min (flow plus) and column temperature [50 ºC (control), 45 ºC (temperature minus), 55 ºC (temperature plus)]. The system suitability criteria of % RSD, theoretical plates and tailing factor was found meeting the criteria in all the conditions.

Solution stability: The standard and sample solutions were injected at different interval up to 24 h by keeping the solution at room temperature. After 24 h, standard solution area differed from initial by 1.1% and sample by 0.1%.

RESULTS AND DISCUSSION

A new stability indicating RP-HPLC method was developed and validated according to ICH guidelines. The system suitability results met the predefined acceptance criteria. No interference was observed at the retention time of MPA from blank, placebo, impurities and all the known impurities are well separated from methylprednisolone acetate (MPA) peak. Peak purity

passes for MPA peak in sample spiked with impurities and all the forced degradation samples confirms that no impurity co-elutes with MPA peak. MPA is degrades in acidic, basic and oxidative stress condition (Table-1). All the degradants are well separated from MPA peak and each other indicating the method is specific and stability indicating. The RSD for the six replicate assay results from method precision experiment found to be 0.2% and RSD for individual known and total impurities are 0.0% indicating the method is precise for the determination of assay and related substances. The overall RSD for the assay and impurities from 12 results (n = 6 from method precision and n = 6 from intermediate precision) found to be 0.9% and 4.1%, respectively indicating the method is rugged and reproducible (Table-2). The % mean accuracy of MPA at each level were ranging from 98.6% to 99.8% for assay with overall mean accuracy of 99.1% (Table-3). The % overall mean recovery from LOQ to 200% for MPA for related substance concentration is found to be 103.9% and % overall mean recoveries of impurities are found to be in the range of 101.4% to 104.8%. The individual and mean recoveries for MPA and impurities were found to be well within the acceptable range of 85% to 115% indicating that the method is accurate in the range of LOQ (0.05%) to 200% of specification limit of impurities and 50% to 150% of test concentration for assay (Table-3). The correlation coefficient of regression line was found to be greater than 0.999 for MPA and all the known impurities indicating the method is linear (Tables 4 and 5). Limit of detection and limit of quantification was demonstrated to be 0.017%w/w and 0.05% w/w, respectively. The %RSD for 6 replicate injections of the precision at LOQ was found to be between 0.9% and 3.4%, which is well within 15% indicating that the method is precise at LOQ level. The signal to noise ratio for MPA and

TABLE-1
FORCED DEGRADATION RESULTS

Name of the stress sample	Condition	Peak purity of MPA peak			Assay (%)	Total degradation (%)
		Purity angle	Purity threshold	Results		
Sample as such (unstressed)	–	0.14	0.23	Pass	100.7	0.13
Acid degradation	Drug product treated with 1.0 N HCl for 24 h at 80 °C	0.15	0.23	Pass	92.8	8.92
Base degradation	Drug product treated 0.2 N NaOH for 4 h at room temp.	0.02	0.24	Pass	93.1	3.09
Oxidation	Drug product treated with 30.0% w/v H_2O_2 for 24 h at 80 °C	0.02	0.24	Pass	92.1	6.54
Thermal degradation	Drug product was exposed at 80 °C for 24 h	0.16	0.23	Pass	101.0	0.21
Photolytic degradation	Drug product was exposed to light for 1.2 Million Lux hours and 200-watt h/Square meter	0.16	0.23	Pass	99.7	0.19

TABLE-2
COMPARISON OF METHOD PRECISION AND INTERMEDIATE PRECISION RESULTS

Set No.	Assay in % of label claim		Impurities in %w/w									
			Impurity-1		Impurity-2		Impurity-3		Max. unknown		Total impurities	
	MP	IP	MP	IP	MP	IP	MP	IP	MP	IP	MP	IP
1	101.9	99.9	0.12	0.12	BQL	BQL	BQL	BQL	BQL	BQL	0.12	0.12
2	102.1	99.8	0.12	0.13	BQL	BQL	BQL	BQL	BQL	BQL	0.12	0.13
3	101.6	100.0	0.12	0.13	BQL	BQL	BQL	BQL	BQL	BQL	0.12	0.13
4	101.5	100.3	0.12	0.13	BQL	BQL	BQL	BQL	BQL	BQL	0.12	0.13
5	101.8	100.4	0.12	0.13	BQL	BQL	BQL	BQL	BQL	BQL	0.12	0.13
6	101.9	100.4	0.12	0.13	BQL	BQL	BQL	BQL	BQL	BQL	0.12	0.13
Mean (n = 6)	101.8	100.1	0.12	0.13	BQL	BQL	BQL	BQL	BQL	BQL	0.12	0.13
% RSD (n = 6)	0.2	0.3	0.0	3.2	NA	NA	NA	NA	NA	NA	0.0	3.2
Mean (n = 12)	101.0		0.12		BQL		BQL		BQL		0.12	
% RSD (n = 12)	0.9		4.1		NA		NA		NA		4.1	

Note: BQL: < 0.05%, MP-Method precision, IP-Intermediate precision

TABLE-3
ACCURACY FOR RELATED SUBSTANCE AND ASSAY METHOD

Accuracy level in % of specification limit	Accuracy for RS method				Accuracy for assay method	
	Mean % recovery				Accuracy level in % of test concentration	Mean % recovery
	MPA	Impurity-1	Impurity-2	Impurity-3		
QL (0.05%)	102.8	102.0	103.4	99.4	–	–
50	105.2	102.2	102.2	109.8	50	98.9
100	107.0	101.2	105.5	103.9	100	99.8
150	101.4	100.1	103.9	105.1	150	98.6
200	103.2	101.3	102.9	105.7	–	–
Overall mean recovery	103.9	101.4	103.6	104.8	Overall mean recovery	99.1

TABLE-4
LINEARITY OF MPA FOR ASSAY AND RELATED SUBSTANCES METHOD

Linearity for assay method				Linearity for RS method				
S. No.	Linearity level w.r.t. test concentration	Conc. (µg/mL)	Area response (AU)	S. No.	Linearity level w.r.t. limit (NMT 0.5%)	Linearity level w.r.t. test conc. (%)	Conc. (µg/mL)	Area response (AU)
1	50	200.624	2365556.0	1	LOQ (10%)	0.05	0.201	2699
2	75	300.244	3500509.5	2	20	0.1	0.401	5010
3	90	359.740	4233814.5	3	40	0.2	0.802	10508
4	100	399.865	4771777.5	4	60	0.3	1.204	15567
5	110	439.990	5227341.0	5	80	0.4	1.605	21064
6	120	480.114	5825397.0	6	200	1	4.012	51183
7	150	599.797	7133641.0	7	1000	5	20.060	253434
				8	2000	10	40.120	511261
				9	4000	20	80.240	1013280
				10	10000	50	200.599	2458624
				11	16000	80	320.959	4006978
				12	20000	100	401.198	4963840
				13	24000	120	481.438	5878700
Correlation coefficient		0.999		1.000				
Regression coefficient		0.999		1.000				
Slope		12114.13		12300.49				
Intercept		-89112.74		7035.63				

TABLE-5
LINEARITY OF IMPURITY-1,2,3 FOR RS

S. No.	Linearity level w.r.t. limit (NMT 0.5%)	Linearity level w.r.t. test concentration (%)	Impurity-1		Impurity-2		Impurity-3	
			Concentration (µg/mL)	Area response (AU)	Concentration (µg/mL)	Area response (AU)	Concentration (µg/mL)	Area response (AU)
1	LOQ (10%)	0.05	0.202	2897	0.203	2554	0.202	2748
2	20	0.10	0.405	6259	0.407	5588	0.404	6128
3	50	0.25	1.012	15422	1.017	13827	1.010	14723
4	80	0.40	1.619	24449	1.627	21916	1.616	23068
5	90	0.45	1.822	27434	1.830	24529	1.818	26116
6	100	0.50	2.024	30700	2.034	27402	2.020	29209
7	110	0.55	2.226	33194	2.237	29665	2.222	31360
8	120	0.60	2.429	37039	2.441	33159	2.425	35300
9	150	0.75	3.036	45756	3.051	40911	3.031	43427
10	200	1.00	4.048	59832	4.068	53467	4.041	56849
Correlation coefficient			1.000		1.000		1.000	
Regression coefficient			1.000		0.999		0.999	
Slope			14864.06		13222.53		14119.78	
Intercept			319.58		291.39		363.15	

TABLE-6
PRECISION AT LOQ, LOD RESULTS

S. No.	Peak area			
	MPA	Impurity-1	Impurity-2	Impurity-3
1	2897	2899	2627	2771
2	2696	2898	2562	2790
3	2711	2892	2538	2744
4	2692	2945	2536	2732
5	2865	2862	2539	2703
6	2700	2885	2523	2749
Mean (n = 6)	2760.167	2896.833	2554.167	2748.167
% RSD (n = 6)	3.4	0.9	1.5	1.1
LOQ conc. (µg/mL)	0.201	0.202	0.203	0.202
LOQ conc. (%w/w)	0.05	0.05	0.05	0.05
S/N	63.4	166.4	89.1	131.5
LOD conc. (µg/mL)	0.066	0.067	0.067	0.067
LOD conc. (%w/w)	0.017	0.017	0.017	0.017
S/N	17.9	48.2	25.9	37.7

all the known impurities in LOD, LOQ solutions were found to be greater than 3 and 10, respectively indicating that the method is sensitive (Table-6). The predefined system suitability criteria were met for all the robustness conditions indicating that the deliberate change in the chromatographic parameters has no significant effect on chromatographic behaviour of the method therefore the method is robust. The standard and sample solution are found to be stable for 24 h at room temperature. The range of the method is defined as 50% to 150% of test concentration for assay and LOQ (0.05%) to 200% of the specification limit for related substances based on demonstrated linearity, accuracy and precision. There is no method available for related substance in USP monograph and newly developed combined assay and related substances method are added advantage to the existing method.

Conclusion

A new stability indicating, mass compatible RP-HPLC method has been developed for the simultaneous estimation of assay, related substance of methylprednisolone acetate (MPA) in methylprednisolone acetate injectable suspension. The developed method is simple, fast, economic, mass compatible, specific, stability indicating, accurate, linear, precise, rugged, sensitive and robust. Hence, this method can be employed for the estimation of assay, content uniformity and related substances in quality control laboratories of pharmaceutical industries.

ACKNOWLEDGEMENTS

The authors express their gratitude to Koneru Lakshmaiah Educational Foundation for the support provided. Authors express their gratitude to S.J.S. Pharmaceutical Labs, Hyderabad, India for providing the necessary research support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. K. Tian, H. Cheng, J. Zhang and K. Chen, *Medicine*, **97**, e0240 (2018); http://dx.doi.org/10.1097/MD.0000000000010240
2. J. Mehta, R. Rolta, B.B. Mehta, N. Kaushik, E.H. Choi and N.K. Kaushik, *Front. Microbiol.*, **13**, 813358 (2022); https://doi.org/10.3389/fmicb.2022.813358
3. L. Zhu, X. Xu, K. Ma, J. Yang, H. Guan, S. Chen, Z. Chen and G. Chen, *Am. J. Transplant.*, **20**, 1859 (2020); https://doi.org/10.1111/ajt.15869
4. FDA Approved Drug Products, Depo-Medrol Methylprednisolone Acetate Injection https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/011757s114lbl.pdf.
5. United States Pharmacopeia 2021, Methylprednisolone Acetate Injectable Suspension Monograph.
6. United States Pharmacopeia 2021, Neomycin and Methylprednisolone Acetate Cream USP Monograph.
7. United States Pharmacopeia 2021, Methylprednisolone Acetate Monograph for Drug Substance.
8. European Pharmacopeia 10.4, Methylprednisolone Acetate Monograph for Drug Substance.
9. K. Tsuji and R.B. Binns, *J. Chromatogr. A*, **253**, 227 (1982); https://doi.org/10.1016/S0021-9673(01)88380-1
10. E.C. Signoretti, L. Valvo, A.L. Savella and G. Cavina, *J. Pharm. Biomed. Anal.*, **11**, 587 (1993); https://doi.org/10.1016/0731-7085(93)80009-P
11. N.K. Hopkins, C.M. Wagner, J. Brisson and T.E. Addison, *J. Chromatogr. A*, **577**, 87 (1992); https://doi.org/10.1016/0378-4347(92)80601-L
12. D.C. Garg, P. Ng, D.J. Weidler, E. Sakmar and J.G. Wagner, *Res. Commun. Chemical Pathol. Pharmacol.,* **22**, 37 (1978).
13. M. Alvinerie and P.L. Toutain, *J. Chromatogr. A*, **309**, 385 (1984); https://doi.org/10.1016/0378-4347(84)80047-X
14. A. Panusa, M. Orioli, G. Aldini and M. Carini, *J. Pharm. Biomed. Anal.*, **51**, 691 (2010); https://doi.org/10.1016/j.jpba.2009.09.041
15. V. Garg and W.J. Jusko, *J. Chromatogr. A*, **567**, 39 (1991); https://doi.org/10.1016/0378-4347(91)80307-X
16. L. Solomun, S. Ibric, V. Vajs, I. Vuckovic and Z. Vujic, *J. Serb. Chem. Soc.*, **75**, 1441 (2010); https://doi.org/10.2298/JSC100115087S
17. J. Li, Y. Liu and Y. Li, *Chinese J. Pharm. Anal.*, **32**, 689 (2012).
18. Z.W. Geng, Y.-J. Yang and W. Liu, *Chinese J. Pharm. Anal.*, **32**, 1093 (2012).
19. International Conference on Harmonization Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1). Current Step 4 version Parent Guideline dated 27 October 1994. (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005).
20. International Conference on Harmonisation, Q1A (R2) Stability Testing of New Drug Substances and Products.
21. L.R. Snyder, J.J. Kirkland and J.L. Glajch, Practical HPLC Method Development, John Wiley & Sons Inc., Ed.: 2 (1997).
22. G.H. Jeffery, J. Bassett, J. Mendham and R.C. Denney, Vogel's Textbook of Quantitative Chemical Analysis, Longman Scientific & Technical, John Wiley & Sons Inc., Ed.: 5 (1989).