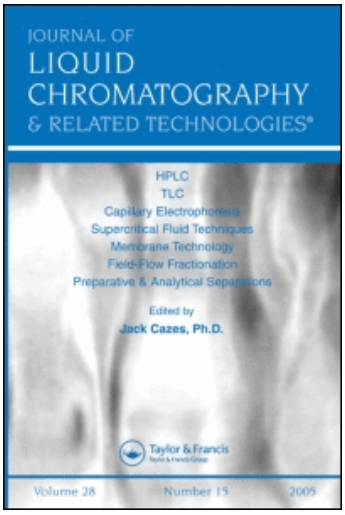

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Development and Validation of a Sensitive and Robust Wipe-Test Method for the Detection and Quantification of the Antibiotic Ertapenem and its Primary Degradates in a Pharmaceutical Manufacturing Environment

Peter Sajonz[a]; Theresa K. Natishan[a]; Yan Wu[a]; Neil T. McGachy[a]; David DeTora[a]
[a] Merck Research Laboratories, Merck & Co., Inc., Rahway, New Jersey, USA

To cite this Article Sajonz, Peter , Natishan, Theresa K. , Wu, Yan , McGachy, Neil T. and DeTora, David(2005) 'Development and Validation of a Sensitive and Robust Wipe-Test Method for the Detection and Quantification of the Antibiotic Ertapenem and its Primary Degradates in a Pharmaceutical Manufacturing Environment', Journal of Liquid Chromatography & Related Technologies, 28: 5, 713 — 725
To link to this Article: DOI: 10.1081/JLC-200048892
URL: http://dx.doi.org/10.1081/JLC-200048892

Journal of Liquid Chromatography & Related Technologies®, 28: 713–725, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1081/JLC-200048892

Development and Validation of a Sensitive and Robust Wipe-Test Method for the Detection and Quantification of the Antibiotic Ertapenem and its Primary Degradates in a Pharmaceutical Manufacturing Environment

Peter Sajonz, Theresa K. Natishan, Yan Wu, Neil T. McGachy, and David DeTora
Merck Research Laboratories, Merck & Co., Inc., Rahway, New Jersey, USA

Abstract: A sensitive HPLC method for the detection and quantification of residual amounts of the 1β-methyl carbapenem antibiotic ertapenem and its primary degradates in swabs collected from manufacturing equipment surfaces was developed and validated. The method utilizes a Waters YMC basic column at ambient temperature, aqueous phosphoric acid and acetonitrile as mobile phases, and UV detection at 230 nm. The method employs gradient elution. The injection precision, linearity, limit of quantitation, limit of detection, selectivity, accuracy, ruggedness, and stability of the method were evaluated and found to be satisfactory. The HPLC method was validated using a swabbing or wipe-test procedure with 4 swabs moistened with 3-(N-morpholino) propane sulfonic acid buffer at pH 7. The limit of quantitation (LOQ) and limit of detection (LOD) were determined to be 0.016 μg/mL (representing 0.16 μg/wipe-test) and 0.0006 μg/mL (representing 0.006 μg/wipe-test), respectively. The solution stability of a 2.0 μg/mL standard solution was evaluated for 18.5 hours at 5°C and found to be satisfactory. The frozen (−20°C) swab stability revealed that the swabs were stable for up to 4 days.

Keywords: Pharmaceuticals, manufacturing, ertapenem, antibiotics, cleaning validation, swab analysis, HPLC

Address correspondence to Peter Sajonz, Merck Research Laboratories, Merck & Co., Inc., RY818-B207, P.O. Box 2000, Rahway, NJ 07065, USA. E-mail: peter_sajonz@merck.com

INTRODUCTION

Wipe test assays find wide application in the detection of chemical and radioactive residues or microorganisms on surfaces.[1–4] In the pharmaceutical industry, these assays are often used (1) to ensure equipment surfaces are clean to avoid potential cross-contamination of a subsequent process, (2) to monitor the workplace to ensure the safety of employees in these areas and (3) to monitor certain process environments to ensure areas maintain acceptable conditions. Since guidance from regulatory agencies, e.g., the Food and Drug Administration (FDA) in the United States or the European Agency for the Evaluation of Medicinal Products (EMEA), specifically discuss the use of wipe tests as part of cleaning verification and validation, such assays for this purpose are common.[5,6] Certain classes of compounds, including penicillins, cephalosporins and other β–lactam antibiotics, are of special concern in pharmaceutical production. The potential medical implications of a cross-contamination event resulting in an unanticipated exposure of a person sensitive to these compounds are significant.[7] Consequently, the need for assays to confirm the absence of such compounds from equipment or processing-area surfaces is clear.

Ertapenem is a novel synthetic broad-spectrum 1β-methylcarbapenem antibiotic. The bulk drug substance ertapenem sodium is a monosodium salt. Ertapenem is the active pharmaceutical ingredient used in the preparation of Invanz™, a trademark of Merck & Co., Inc., Whitehouse Station, NJ, USA. Invanz™ is used for the treatment of adult patients with moderate to severe infections, e.g., complicated intra-abdominal infections, community acquired pneumonia, and complicated urinary tract infections, that are caused by specific strains of susceptible microorganisms.[8–12] The ertapenem molecule (Figure 1) consists of a carbapenem ring and a side chain. Hydrolysis of the highly strained ring system accounts for the instability of carbapenem antibiotics in water at high and low pH and leads to the ring-opened hydrolysis

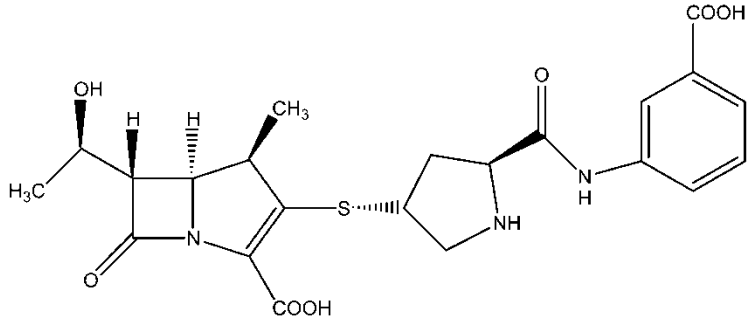

Figure 1. Molecular structure of ertapenem.

degradate. In addition to the hydrolysis degradate, other degradates appear in aqueous solution. These degradates tend to form at higher ertapenem concentrations. These impurities are dimers and dehydrated dimers of ertapenem.[13] This instability of ertapenem is a very important issue that needs to be addressed appropriately during the development of a suitable wipe-test procedure. Residual ertapenem on a given manufacturing surface will have degraded to some extent and this has to be considered during the analysis and calculation of the reported results, i.e., the quantification of the total amount of residual antibiotic has also to account for the major degradates. Furthermore, degradation has to be prevented during all stages of the wipe-test method, starting from the wiping procedure to the sample preparation and final analysis.

The goal of this work was the development and validation of a sensitive and robust wipe-test method for the detection and quantification of residual ertapenem and its primary degradates, the ring-opened hydrolysis product and dimers.

EXPERIMENTAL

Chemicals

Ertapenem sodium reference standard samples were supplied by Merck Sample Repository (Merck Research Laboratories, Rahway, New Jersey, USA). The water used was distilled and purified by a HYDRO System (Garfield, NJ, USA). Sodium hydroxide (50%) and ortho phosphoric acid (85%) were purchased from Fisher Scientific (Fisher Scientific, Fair Lawn PA, USA). Acetonitrile was obtained from EM Science (Gibbstown, NJ, USA). MOPS (3-(N-morpholino) propane sulfonic acid, SigmaUltra grade, $pK_a = 7.2$, purity $> 99.5\%$) was obtained from Sigma (St.Louis, MO, USA), MES (4-morpholineethane sulfonic acid monohydrate, $pK_a = 6.1$, purity 98%) and EPPS (4-(2-hydroxyethyl)-1-piperazine-propanesulfonic acid, $pK_a = 8.0$, purity 99%) were purchased from Aldrich (St.Louis, MO, USA).

Materials

Swabs (Alpha Swab with Long Handle, TX761) that were used for wiping surfaces were purchased from Texwipe, Upper Saddle River, NJ, USA. The conical tubes (Falcon 352095, 15 mL polystyrene conical tubes) that were used for the extraction procedure after wiping were obtained from Becton Dickinson, Franklin Lakes, NJ, USA. The surfaces used to test the wipe-test method recovery consisted of a stainless steel coupon and a Pyrex glass plate.

Table 1. Experimental system and conditions for the HPLC method

Time (min)	%A	%B
Gradient profile (including equilibration)		
0	90	10
15	60	40
16	10	90
19	10	90
20	90	10
30	90	10

HPLC system: Agilent 1100 (Agilent Technologies, Palo Alto, CA, USA).

Column: YMC basic, 10 × 0.46 cm, (Waters, Milford, MA, USA).

Flow rate: 1.5 mL/min.

Injection volume: 100 μL.

Sample tray temperature: 5°C.

Column temperature: ambient.

Mobile phase: A: 0.05% phosphoric acid in water, B: acetonitrile.

Equipment

An Agilent 1100 Series HPLC system equipped with an auto injector, a sample tray cooler, a quaternary pump, a column oven, and a diode array detector (Agilent Technologies, Palo Alto, CA, USA) was used for analytical analyses of wipe samples. A gradient HPLC method was used. The chromatographic conditions are shown in Table 1. The method was able to separate the main component ertapenem, the hydrolysis degradates, and the dimeric degradates. The wavelength of the detector was set to 230 nm. At this wavelength, the response of the detector was linear in the concentration range that was used.

Preparation of Materials

The buffer solutions were prepared by dissolving 10 mM of MOPS, MES, or EEPS into 1 L water. The solutions were titrated to pH 7.0, 5.5, or 9 with aqueous sodium hydroxide solution. The swab blank was prepared by placing 4 swabs in a tapered 15 mL centrifuge tube, adding 10 mL (using a volumetric pipet) of MOPS diluent, then vortexing or vigorously shaking the sample. Ertapenem standard solutions were prepared using ertapenem monosodium reference standard and 10 mM MOPS diluent at pH 7.

A working standard of 0.5 μg/mL was used for quantitation of ertapenem and degradates in the swab samples and a 2.0 μg/mL standard solution to observe where the ring opened and dimer impurities elute.

The wipe samples were prepared by extracting the swabs with 10 mL MOPS buffer in a tapered 15 mL centrifuge tube.

Procedures

The recovery studies were performed by spotting a surface, i.e., stainless steel or glass, with a known volume of an ertapenem solution of known concentration. After the solvent was evaporated under a nitrogen stream, the spot was wiped with a swab dampened with MOPS diluent, followed by a second dry swab, followed by a third dampened swab, and followed by a fourth dry swab. The four swabs were placed into a 15 mL conical tube and 10 mL of MOPS diluent pipetted into the tube. The tube was shaken vigorously and then a portion of the solution was analyzed. The amount of ertapenem recovered was compared with the amount actually spotted on the surface to calculate the recovery.

RESULTS AND DISCUSSION

HPLC and Wipe-Test Method Development

The HPLC method was designed to allow for simple operation and implementation, e.g., to test for residual ertapenem and its major degradates in a manufacturing environment. Gradient elution and an injection volume of 100 μL were used to enhance sensitivity. The choice of the monitoring UV wavelength of 230 nm was made because this wavelength corresponds to the location of the adsorption maximum of the carbapenem ring system of the ertapenem molecule, thus further optimizing the selectivity and sensitivity of the method for carbapenem antibiotics. The HPLC method was established with the conditions shown in Table 1. Typical chromatograms of a MOPS blank (MOPS diluent at pH 7), swab blank (4 swabs with 10 mL of MOPS diluent), ertapenem standard solution, and recovered ertapenem solution from a stainless steel coupon are shown in Figure 2. Ertapenem elutes at 8.4 minutes and is well separated from the ring opened degradate and dimers, which elute at 6.3 and 11–12 min, respectively.

Various non-nucleophilic buffers, e.g., MOPS (pH = 7), EPPS (pH 9.5), and MES (pH 5.5), were investigated to find the best stability of ertapenem in solution. Non-nucleophilic buffers have been chosen to avoid a nucleophilic attack resulting in opening of the carbapenem ring system.[14,15] The results

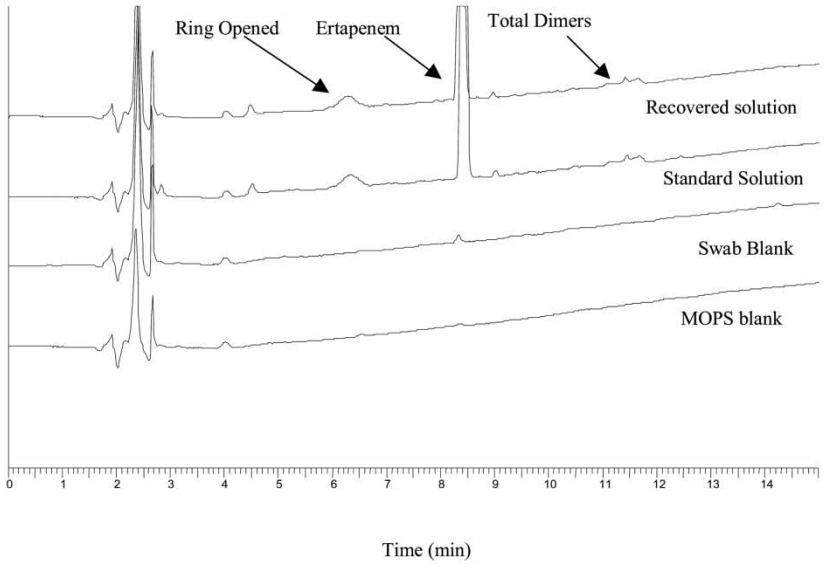

Figure 2. Chromatograms of MOPS blank, swab blank, 2.0 μg/mL ertapenem standard solution and 2.0 μg/mL recovered standard from stainless steel coupon.

of this stability study are shown in Figure 3. It can be seen that MOPS buffer at a neutral pH of 7 provides for an optimal stability of ertapenem solution. At higher pH, it is predominantly the opening of the β-lactam ring that contributes to the degradation. MOPS buffer at pH 7.0 was, therefore, chosen as diluent for the wipe-test method. It provides a pH neutral diluent to enhance the stability of the ertapenem wipe samples and extracted ertapenem solutions. Although MES at pH 5.5 shows comparable stability to MOPS at pH 7, a neutral pH is preferred since at higher ertapenem concentrations and lower pH, dimer formation can occur.[13]

The wiping technique, using 4 swabs as described in the experimental section, was designed to ensure good sample recovery while minimizing sample dilution. The dilution during the extraction procedure is only about 10x and the swabs used (Alpha Swab TX761) provided a clean baseline without significant interference.

Validation of the HPLC Method

The HPLC method was validated by determining the injection precision, linearity, limits of detection and quantification, selectivity, and solution stability. The wiping technique was validated by examining the accuracy

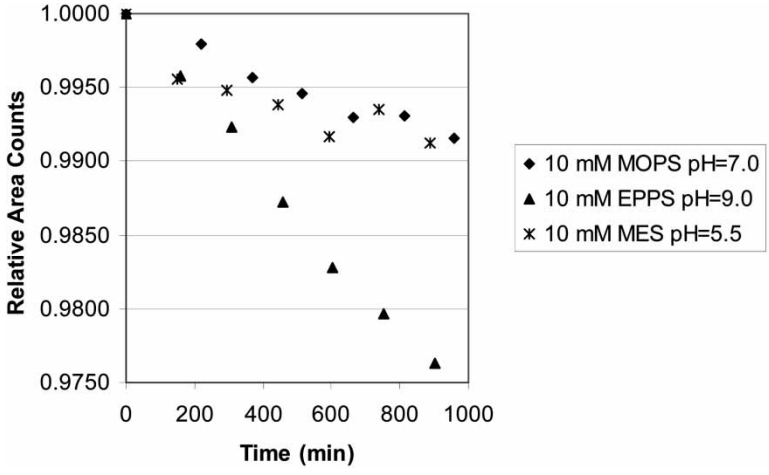

Figure 3. Stability of ertapenem at 5°C in aqueous solution at a diluted concentration of 0.2 g/L. Change of relative area counts versus time for three buffers used: MOPS (pH 7), MES (pH 5.5) and EEPS (pH 9.5).

and ruggedness and the stability of frozen swab samples. The validation was performed consistent with expectations for use of the wipe-test method in a pharmaceutical manufacturing environment.[16,17]

Injection precision was demonstrated by determining the %RSD based on the area counts of ertapenem and its primary degradates of six injections of a 2.0 μg/mL reference standard solution. The %RSD based on the area counts of ertapenem, ring opened, and total dimers was 0.1%, 10.2%, and 9.9%, respectively (see Table 2). This demonstrated satisfactory injection precision.

Table 2. Injection precision of a 2.0 μg/mL ertapenem standard solution

Injection #	Ertapenem area counts	Ring opened area counts	Total dimers area counts
1	357,492	5,955	3,882
2	358,162	5,997	3,841
3	357,768	6,168	3,212
4	357,336	6,843	3,853
5	357,139	6,156	3,902
6	357,379	7,635	3,139
Average	357,546	6,459	3,638
Standard Deviation	366	659	360
% RSD	0.10	10.2	9.9

Linearity of the detector response for ertapenem was evaluated over the concentration range of 0.002 to 9.867 μg/mL (representing 0.02 to 98.7 μg/wipe-test). Solutions of ertapenem reference standard were prepared using serial dilutions. Three injections were made at each concentration. The detector response of ertapenem was found to be linear over the entire range (see Table 3). The regression coefficient R^2 was 0.998; this is satisfactory considering the wide concentration range investigated.

The limit of quantification (LOQ) and limit of detection (LOD) were determined by preparing serial dilutions and triplicate injections. The LOD for ertapenem was established by determining the lowest concentration at which ertapenem has a signal to noise ratio of 3 to 1. The LOQ was established as the concentration of ertapenem that satisfied the following requirements, i.e., (1) a minimum signal to noise (S/N) ratio of 10 to 1, (2) a maximum deviation of 20% between the response factor obtained at the LOQ and the response factor of the ertapenem standard solution which is five times more concentrated, and (3) a maximum RSD of 15% for the area counts of repeated injections at the LOQ. The LOQ was found to be 0.016 μg/mL

Table 3. Linearity of ertapenem in MOPS solution at 230 nm

Concentration (μg/mL)	Area counts ertapenem
9.867	2,406,719
	2,264,080
	2,395,760
1.974	419,423
	484,149
	461,782
0.987	173,141
	241,258
	240,969
0.197	43,818
	29,592
	20,096
0.040	8,686
	6,754
	5,127
0.008	2,161
	2,212
	2,205
0.002	527
	518
	541
$R^2 = 0.998$	

(representing 0.16 μg/wipe-test) and the LOD 0.0006 μg/mL (representing 0.006 μg/wipe-test), see Table 4.

Method selectivity was demonstrated by injecting the 10 mM MOPS pH 7.0 diluent and the 2.0 μg/mL standard solution (see Figure 2). The 10 MOPS diluent did not cause any significant chromatographic interference with ertapenem, ring opened, and total dimer degradates.

Solution stability was tested at 5°C by performing consecutive injections of the 2.0 μg/mL reference standard solutions held in the auto-sampler tray cooler. Table 5 gives the area counts for ertapenem, ring opened, and total dimers for up to 18.5 hours. The solution stability was found to be satisfactory for up to 18.5 hours with the %RSD's of ertapenem, ring opened, and total dimers of 0.3%, 10.5%, and 12.6%, respectively. The % change in the area counts is also shown in Table 5. The % change after 18.5 h is −0.4% for

Table 4. Determination of the LOD and LOQ for ertapenem

Ertapenem concentration (μg/mL)	Area counts	Average area Cts	%RSD	rf*	%dev of rf**	S/N	
2.0	353,168	3,53,006	0.12	1,76,503	—		
	352,512						
	353,337						
0.4	72,371	72,554	0.24	1,81,385	2.8	950.0	
	72,711						
	72,580						
0.08	14,730	14,924	2.18	1,86,550	2.8	223.2	
	15,300						
	14,742						
0.016	3,175	3,183	1.32	1,98,938	6.6	54.4	LOQ
	3,146						
	3,229						
0.0032	792	778	3.23	2,43,125	22.2	10.9	
	749						
	793						
0.00064	246	261	5.15	4,07,813	67.7	6.3	LOD
	272						
	265						
0.000128	174	217	17.4	16,95,313	315.7	2.9	
	231						
	245						

*rf = response factor.

**%deviation of rf is calculated as $100(rf - rf_{5x})/rf_{5x}$ where rf_{5X} is the response factor of the 5x more concentrated solution.

Table 5. Solution stability of ertapenem in MOPS pH 7 buffer at a concentration of 2.0 μg/mL

	Ertapenem		Ring opened		Total dimers	
Time (hours)	Area counts	Change (%)	Area counts	Change (%)	Area counts	Change (%)
0	357,492	0.00	5,955	0.00	3,882	0.00
0.5	358,162	0.19	5,997	0.71	3,841	−1.06
1.0	357,768	0.08	6,168	3.58	3,212	−17.26
1.5	357,336	−0.04	6,853	15.08	3,853	−0.75
2.0	357,139	−0.10	6,156	3.38	3,902	0.52
2.5	357,379	−0.03	7,635	28.21	3,139	−19.14
4.0	356,621	−0.24	6,560	10.16	3,637	−6.31
6.5	356,605	−0.25	6,548	9.96	3,488	−10.15
12.0	354,231	−0.91	7,732	29.84	3,277	−15.58
12.5	354,566	−0.82	7,570	27.12	3,092	−20.35
13	356,173	−0.37	7,537	26.57	3,772	−2.83
14.5	356,239	−0.35	7,818	31.28	3,083	−20.58
16.0	357,008	−0.14	7,691	29.15	2,676	−31.07
18.5	355,988	−0.42	7,563	27.00	2,719	−29.96
Average	356,622		6,985		3,398	
Standard deviation	1,131		730		429	
%RSD	0.3		10.5		12.6	

ertapenem, 27% for ring opened, and −30% for total dimers. These values indicate that ertapenem and dimers degrade in solution while ring opened is formed over time. The %RSD and % change results of ring opened and total dimers are large due to the low area counts present at the standard concentration level.

Validation of the Swab Wiping Technique

Accuracy and ruggedness of the method were determined by recovery studies at both 0.5 and 2.0 μg concentration levels. The two surfaces studied were stainless steel and glass. The data shown in Table 6 reveal that recoveries of ≥95% were obtained on each surface at each spotting level. The analyses were conducted by two different analytical chemists. The results are reproducible. Table 5 shows the comparison of the recovery data between the two chemists.

The stability of swabs at frozen storage conditions was evaluated at 0.5 and 2.0 μg per swab. After spotting and wiping either the 0.5 or 2.0 μg of

Table 6. Recovery of ertapenem from stainless steel coupon and glass surface

Surface	Level of ertapenem	Recovery (%)	Analytical chemist
Stainless steel	0.5 μg/wipe-test	98	PS
		100	PS
		99	PS
		101	TKN
		101	TKN
		100	TKN
Glass	0.5 μg/wipe-test	97	PS
		95	PS
		99	PS
		98	TKN
		97	TKN
		98	TKN
Stainless steel	2.0 μg/wipe-test	102	PS
		100	PS
		99	PS
		100	TKN
		99	TKN
		98	TKN
Glass	2.0 μg/wipe-test	98	PS
		100	PS
		104	PS
		97	TKN
		97	TKN
		98	TKN

reference standard from the stainless steel coupon, the swabs were placed in the conical polystyrene tubes without adding MOPS diluent and stored immediately at −20°C. The samples were analyzed over a 4 day time period and the recoveries were compared (Table 7). The results indicate

Table 7. Frozen (−20°C) stability of the ertapenem swab samples recovered from stainless steel coupon

	% Recovery	
Time-point	0.5 μg/wipe-test	2.0 μg/wipe-test
Initial	101	97
1 day	96	95
2 days	99	100
4 days	96	98

that the frozen swabs are stable with recoveries $\geq 95\%$ for up to 4 days at frozen storage conditions.

CONCLUSIONS

A fast, robust, and sensitive wipe test method for the detection and quantification of residual amounts of the antibiotic ertapenem and its primary degradates on manufacturing surfaces was developed and validated. The method employs gradient HPLC with UV detection. The injection precision, linearity, limit of quantitation, limit of detection, selectivity, accuracy, ruggedness, and stability were evaluated and found to be satisfactory. The method can be used routinely to detect and quantify residual levels of ertapenem and its primary degradates on various surfaces to ensure cleanliness of manufacturing equipment and a safe work environment.

ACKNOWLEDGMENTS

We gratefully acknowledge our colleagues at Merck Research Laboratories for their support in the present study.

REFERENCES

1. Nozal, M.J.; Bernal, J.L.; Toribio, L.; Martin, M.T.; Diez, F.J. J. Pharm. Biomed. Anal. **2002**, *30*, 285.
2. Nozal, M.J.; Bernal, J.L.; Toribio, L.; Martin, M.T.; Diez, F.J. J. Chromatogr. A **2001**, *919*, 87.
3. Nozal, M.J.; Bernal, J.L.; Toribio, L.; Jimenez, J.J.; Martin, M.T. J. Chromatogr. A **2000**, *870*, 69.
4. Sottani, C.; Turci, R.; Micoli, G.; Fiorentino, M.L.; Minoia, C. Rapid Commun. Mass Spectrom. **2000**, *14*, 930.
5. *Good Manufacturing Practices for Active Pharmacuetical Ingredients*; Vol. 4, Annex 18; EudraLex: section 12.73.
6. *Guide to Inspections of Validation of Cleaning Processes*; Food and Drug Administration: Washington DC, USA, 1993.
7. Beta-Lactam, Antibiotics. Anaphylaxis. J. Allergy Clin. Immunol. **1998**, *10* (1), S498–S499.
8. *Prescribing Information for Invanz*; Merck & Co., Inc.: Whitehouse Station, NJ, 2003.
9. Cunha, B. Drugs of Today **2002**, *38*, 195.
10. Mouton, J.; Touw, D.; Horrevorts, A.; Vinks, A. Clin. Pharmacokinet. **2000**, *39*, 185.
11. Sader, H.; Gales, A. Drugs **2001**, *61*, 553.
12. Wong, B.; Bruhin, P.; Lin, J. J. Pharm. Sci. **1999**, *88*, 277.

13. Sajonz, P.; Natishan, T.; Wu, Y.; Williams, J.; Pipik, B.; Dimichele, L.; Novak, T.; Pitzenberger, S.; Dubost, D.; Almarsson, O.J. Liq. Chroma. & Rel. Technol. **2001**, *24*, 2999.
14. Vilanova, B.; Munoz, F.; Donoso, J.; Blanco, F.G. Helv. Chim. Acta **1993**, *76*, 2789.
15. Smith, G.; Schoenewaldt, E.J. Pharm. Sci. **1981**, *70*, 272.
16. International Conference for Harmonization, 1995. Guidance for Industry; ICH Q2A Text on Validation of Analytical Procedures.
17. International Conference for Harmonization, 1996. Guidance for Industry; ICH Q2B Validation of Analytical Procedures: Methodology.

Received September 23, 2003
Accepted October 18, 2003
Manuscript 6497