Journal of Chromatographic Science, Vol. 49, August 2011

# A Validated Stability-Indicating RP-HPLC Assay Method for Amsacrine and its Related Substances

**M. Lalitha Devi[1,*], K.B. Chandrasekhar[2], K.V. Surendranath[3], B.M. Rao[4],** and **M.B.V. Narayana[5]**

[1]Reference Standard Laboratory, United States Pharmacopeia-India Private Limited, ICICI Knowledge Park, Turkapally, Shameerpet, and Hyderabad 500 078, India; [2]Department of Chemistry, Jawaharlal Nehru Technological University. Anantapur 515002, India; [3]Reference Standard Laboratory, United States Pharmacopeia-India Private Limited, ICICI Knowledge Park, Turkapally, Shameerpet, and Hyderabad 500 078, India; [4]Jhonson Cilag, Johnson and Johnson Pharma Mumbai 400 036, India; and [5]Matrix Laboratories Ltd.Hyderabad, 500055, India

## Abstract

**A validated specific stability indicating reversed-phase high-performance liquid chromatography method was developed for the quantitative determination of Amsacrine as well as its related substances determination in bulk samples, in presence of degradation products, and its process related impurities. Forced degradation studies were performed on bulk samples of Amsacrine as per International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use (ICH) prescribed stress conditions using acid, base, oxidative, thermal stress, and photolytic degradation to show the stability indicating power of the method. Significant degradation was observed during basic hydrolysis, slight degradation was observed in oxidative and thermal stress, and no degradation was observed in other stress conditions. The chromatographic method was optimized using the samples generated from forced degradation studies and the impurity spiked solution. Good resolution between the peaks corresponds to process-related impurities and degradation products from the analyte were achieved on Inertsil ODS column using the mobile phase consists a mixture of 1.0% triethyl amine in 20 mM potassium dihydrogen orthophosphate, with pH adjusted to 6.5, with ortho phosphoric acid in water and acetonitrile using a simple linear gradient. The detection was carried out at wavelength 248 nm. The mass balance in each case was in between 99.4% to 99.9%, indicating that the developed method was stability-indicating. Validation of the developed method was carried out as per ICH requirements. The developed method was found to be suitable to check the quality of bulk samples of Amsacrine at the time of batch release and also during its stability studies**

## Introduction

Amsacrine is described chemically as: N-(4-(acridin-9-ylamino)-3-methoxyphenyl)methanesulfonamide. The drug is an antineoplastic agent, which inhibits and combats the development of tumors. Its planar fused ring system can intercalate into the DNA of tumor cells, thereby altering the major and minor groove proportions. Amsacrine also inhibits topoisomerase II activity lead to S phase and G2 arrest (1,2) and may exert an effect on cell membranes (3). This drug also possesses immunosuppressive and antiviral properties (1) and is a chemotherapy drug used to treat some types of cancers including acute adult leukaemia (4).

According to current good manufacturing practices all drugs must be tested with a stability indicating assay method before release. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used. An liquid chromatography method has been developed for the determination of amsacrine (AMSA) in human nucleated hematopoietic cells was available in literature (5,6). To our knowledge, no stability indicating high-performance liquid chromatography (HPLC) method for the quantification of Amsacrine is available in the literature. The present research work was to develop a suitable stability indicating HPLC method for the determination of Amsacrine, and the developed method was validated with respect to specificity, limit of detection (LOD) and quantitation (LOQ), linearity, precision, accuracy, and robustness to show the stability indicating power of the method and also to ensure the compliance in accordance with ICH Guidelines (7). The developed method was applied for analysis of drug stored at long term and accelerated stability studies to show stability indicating of the method.

## Experimental

### Chemicals and reagents

Samples of Amsacrine and its three process impurities (Figure 1) were received from Bulk Actives (Aurobindo pharmaceuticals Ltd, Hyderabad, India). HPLC-grade acetonitrile was purchased from Rankem (Mumbai, India). Potassium dihydrogen orthophosphate was purchased from Qualigens Fine Chemicals (Mumbai, India). Triethylamine was purchased from Loba Chemie (Mumbai, India). High-purity water was prepared by using Millipore Milli Q plus purification system.

*Author to whom correspondence should be addressed: email mullapudilalitha@yahoo.co.in.

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission.

### Chemical names

(*i*) Amsacrine: N-(4-(acridin-9-ylamino)-3-methoxyphenyl) methane sulfonamide (Mol.Wt.: 393.12); (*ii*) Imp-1: N-(4-amino-3-methoxyphenyl) methane sulfonamide (Mol. Wt.: 216.22); (*iii*) Imp-2: Acridin-9(8aH)-one (Mol. Wt.: 195.11); and (*iv*) Imp-3: 9-chloroacridine (Mol. Wt.: 213.03)

### Equipment

The HPLC method development, validation, and forced degradation studies were done using a Waters HPLC system with diode array detector (Waters Chromatography Division, Milford, MA). The data were collected and the peak purity of the Amsacrine peak was checked using Empower-millennium software (Waters Chromatography Division, Milford, MA). Stability studies were carried out in a humidity chamber (Thermo Lab, India) and photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (MACK Pharmatech, Hyderabad, India).

### Chromatographic conditions

The chromatographic separations were achieved on an Inertsil (GL Sciences Inc., Tokyo, Japan) ODS column (150 mm length × 4.6 mm i.d. with 5 µm particle size) using the solvent A as 20 mM potassium dihydrogen orthophosphate with 1.0% Triethyl amine, with pH adjusted to 6.5 with ortho phosphoric acid in water and acetonitrile in the ratio of 90:10 % v/v and solvent B as acetonitrile with a gradient program: time (min) / %B, 0/15, 15/30, 30/80, 35/80 at a flow rate of 1.0 mL/min with a post run time of 5 min. The column temperature was maintained at 25°C, and the detection was carried out at 248 nm. The test concentration was ~ 300 µg/mL, and the injection volume was 20 µL for related substances and 10 µL for assay determination. A degassed mixture of water and acetonitrile in the ratio of 50:50% v/v was used as diluent during the standard and test samples preparations. The degassing was done by using a Supelco Mobile phase degassing system.

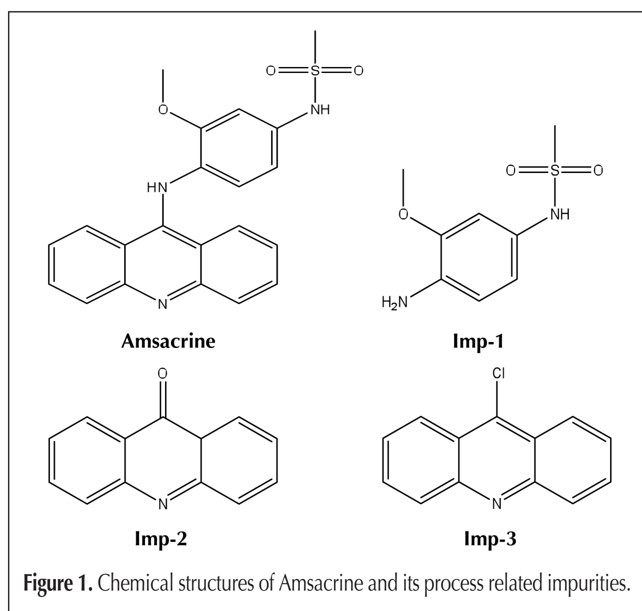


**Figure 1.** Chemical structures of Amsacrine and its process related impurities.

### Preparation of standard solutions

Standard and sample solutions of Amsacrine were prepared separately at ~ 300 µg/mL in diluent for the determination of assay and related substances analysis. A stock solution of impurities (imp-1, imp-2, and imp-3) at 300 µg/mL concentration was prepared in diluent.

## Method Validation

### Stress studies/specificity

Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities/degradation products (8). All stress degradation studies were performed at an initial drug concentration of 300 µg/mL. Acidic stress was performed in 0.5 N HCl at 60°C for 5 days. The study in basic solution was carried out in 0.001 N NaOH at ambient temperature for 10 h. Oxidation studies were carried out at ambient temperature in 3% hydrogen peroxide for 3 days. Photo degradation studies were carried out according to Option 2 of Q1B in International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use (ICH) guidelines (9). The drug sample was exposed to light for and overall illumination of 1.2 million lux h and an integrated near ultraviolet energy of 200 W h m2 . The drug sample was exposed to dry heat at 80°C for 10 days. Samples were withdrawn at appropriate times and subjected to LC analysis after suitable dilution (300 µg/mL) to evaluate the ability of the proposed method to separate Amsacrine from its degradation products. Photodiode array detector was employed to ensure the homogeneity and purity of Amsacrine peak in all the stressed sample solutions. Assessment of mass balance in the degraded samples was carried out to confirm that the amount of impurities detected in stressed samples matched with the amount present before the stress was applied. Quantitative determination of Amsacrine was carried out in all the stressed samples against qualified working standard and the mass balance (% assay + % sum of all impurities + % sum of all degradation products) was tabulated in Table I.

### LOD and LOQ

The LOD and LOQ for imp-1, imp-2, and imp-3 were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, (10, 11) by injecting a series of diluted solutions with known concentrations. Precision study was also carried at the LOQ level by injecting six individual preparations of imp-1, imp-2, and imp-3 and calculating the RSD percentage of the area.

### Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample (12). Linearity test solutions for the assay method were prepared from 50% to 150% with respect to analyte concentration of 300 µg/mL

(i.e. 150, 225, 300, 375, and 450 µg/mL), respectively. The peak area versus concentration data was performed by least-squares linear regression analysis. Linearity test solutions for related substance methods were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% with respect to the impurities specification level of 0.10% (i.e., LOQ, 0.05, 0.075, 0.10, 0.125, 0.15, and 0.2%). The calibration curve was drawn by plotting the peak areas of imp-1, imp-2, and imp-3 versus its corresponding concentration. Linearity test was performed for two consecutive days in the same concentration range for both assay and related substance method. The correlation coefficient of the calibration curve was calculated.

### Precision

Assay method precision was evaluated by carrying out six independent assays of test samples of Amsacrine against qualified working standard and calculated the percentage of RSD. The precision of the related substance method was checked by injecting six individual preparations of Amsacrine spiked with 0.10% level of imp-1, imp-2, and imp-3 with respect to target analyte concentration (i.e., 300 µg/mL). RSD percentage for imp-1, imp-2, and imp-3 was calculated. The intermediate precision of the method was also verified using different analyst, different day, and different instruments in the same laboratory.

### Accuracy

The accuracy of an analytical procedure expresses as the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found (13). The accuracy of the assay method was evaluated in triplicate at three concentration levels (i.e. 150, 300, and 450 µg/mL) in bulk drug samples. The percentage recoveries were calculated from the slope and Y-intercept of the calibration curve. The bulk samples available for development work, do not show the presence of imp-1, imp-2, and imp-3. Standard addition and recovery experiments were conducted to determine the accuracy of the related substance method for the quantification of all three impurities in bulk drug samples. The study was carried out in triplicate by spiking each impurity at 0.05, 0.10, and 0.15% in bulk drug sample solution (300 µg/mL). The percentage recoveries for imp-1, imp-2, and imp-3 were calculated from the slope and Y-intercept of the calibration curve

**Table I. Results of stress studies**

| Stress condition | Period of Study | % assay Amsacrine | Mass balance* | Remarks |
|---|---|---|---|---|
| 0.5 N HCl (acidic) | 5 days | 99.8 | 99.7 | No degradation products formed |
| 0.001 N NaOH (Basic) | 10 hours | 76.4 | 99.4 | One Major degradation product formed at 1.22 RRT† |
| 3% $H_2O_2$ (Oxidative) | 3 days | 97.4 | 99.8 | Two Major degradation Products formed at 0.42, 0.74 RRT |
| Thermal degradation at 80°C | 10 days | 99.1 | 99.5 | Mild degradation was observed |
| Photolytic Degradation | 11 days | 99.8 | 99.9 | No degradation products formed |

* (% assay+% impurities +%degradnats).
† RRT-relative retention time.

### Selectivity

The selectivity of the method was established from the resolution of the Amsacrine from the nearest peak and also among all the other peaks. All the degradants and impurities were separated amongst as well as from analyte with a resolution greater than 12 show the selectivity of the method.

### Solution stability and mobile phase stability

The solution stability of Amsacrine was carried out by leaving the test solution in a tightly capped volumetric flask at room temperature for 48 h. The solution was assayed at 6 h intervals to the end of the study period, using a freshly prepared standard solution of Amsacrine for comparison each time. The mobile phase stability was also investigated by assaying the freshly prepared sample solutions against freshly prepared standard solutions at 6 h intervals up to 24 h. Mobile phase composition and preparation was kept constant during the study period. The % RSD of the assay of Amsacrine was calculated during the duration of the mobile phase and solution stability experiments.

### Robustness

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between closely eluting impurity (i.e., imp-2) and Amsacrine was evaluated.

To study the effect of flow rate on the resolution, the flow rate was altered by 0.2 units (i.e. 0.8 and 1.2 mL/min) from the actual flow of 1.0 mL/min. The effect of column temperature on resolution was studied at 20°C and 30°C instead of 25°C. All the other mobile phase components were held constant as described earlier. The effect of pH was studied by changing pH by 0.5 units from the value of 6.5 and keeping the remaining method conditions constant.

## Results and Discussion

### Development and optimization of HPLC method

The main target of the chromatographic method is to get the separation of imp-1, imp-2, imp-3, and the degradation products generated during stress studies from the analyte peak. As the pKa value of Amsacrine was 7.4, potassium dihydrogen orthophosphate buffer with pH 6.5 adjusted with KOH as solvent A and acetonitrile as solvent B at 1.0 mL/min was chosen for the initial trail, with a 150 mm length × 4.6 mm i.d. column and 5 µm particle size C18 stationary phase. When an impurity spiked sample solution was injected, the resolution between impurities and analyte was poor and the retention of amsacrine was reduced. To obtain good resolution of impurities from the analyte and to increase the retention, to the phosphate buffer 1.0 mL of triethyl

amine was added and the pH was adjusted to 6.5 using phosphoric acid. At these conditions, the impurities were separated and stressed solutions were injected. The degradation product formed at 0.74 RRT in oxidative hydrolysis was not well separated from imp-2 at RRT 0.70 although it was separated from main analyte. Separation of imp-2 (0.70 RRT) and degradation product (0.74 RRT) was achieved when solvent A was mixed with acetonitrile in the ratio of 90:10 v/v with some change in gradient program. At these chromatographic conditions, all the impurities and degradants were well separated amongst and also from amsacrine. The effect of buffer pH was also studied under the described conditions, and it was found that at higher and lower pH the tailing of the amsacrine peak was greater, and also the resolution was poor between impurities and degradants and also from the analyte. The results clearly indicated that on Inertsil ODS (150 mm length × 4.6 mm i.d. with 5 µm particle size) and using solvent A (20 mM potassium dihydrogen orthophosphate with 1.0% triethyl amine with pH adjusted to 6.5 with phosphoric acid in water and acetonitrile in the ratio of 90:10% v/v) and solvent B (acetonitrile with a gradient programme: T/%B: 0/15, 15/30, 30/80, 35/80 with a post run time of 5 min at detection wavelength 248 nm) was successful in separation of amsacrine from its impurities and degradation products. Under the described conditions, results were as follows, retention time of amsacrine was around 16.9 min with a tailing factor of 1.02, number of theoretical plates (N) for the amsacrine peak was 45256, % RSD for 5 replicate injections was 0.2, and all the impurities were well separated with a resolution greater then 14 from the Amsacrine peak (Figure 2B). Peak purity of stressed samples of amsacrine was checked by using a photodiode array detector of water with millennium software (Waters, Milford, MA), the purity threshold is greater than purity angle in all the stress samples, demonstrating the homogeneity of analyte peak. Accelerated and long term stability study results as per ICH Q1A (R2) for Amsacrine were generated for 6 months by using the developed LC method and the results were well within the limits this further confirms the stability indicating of the developed LC method.

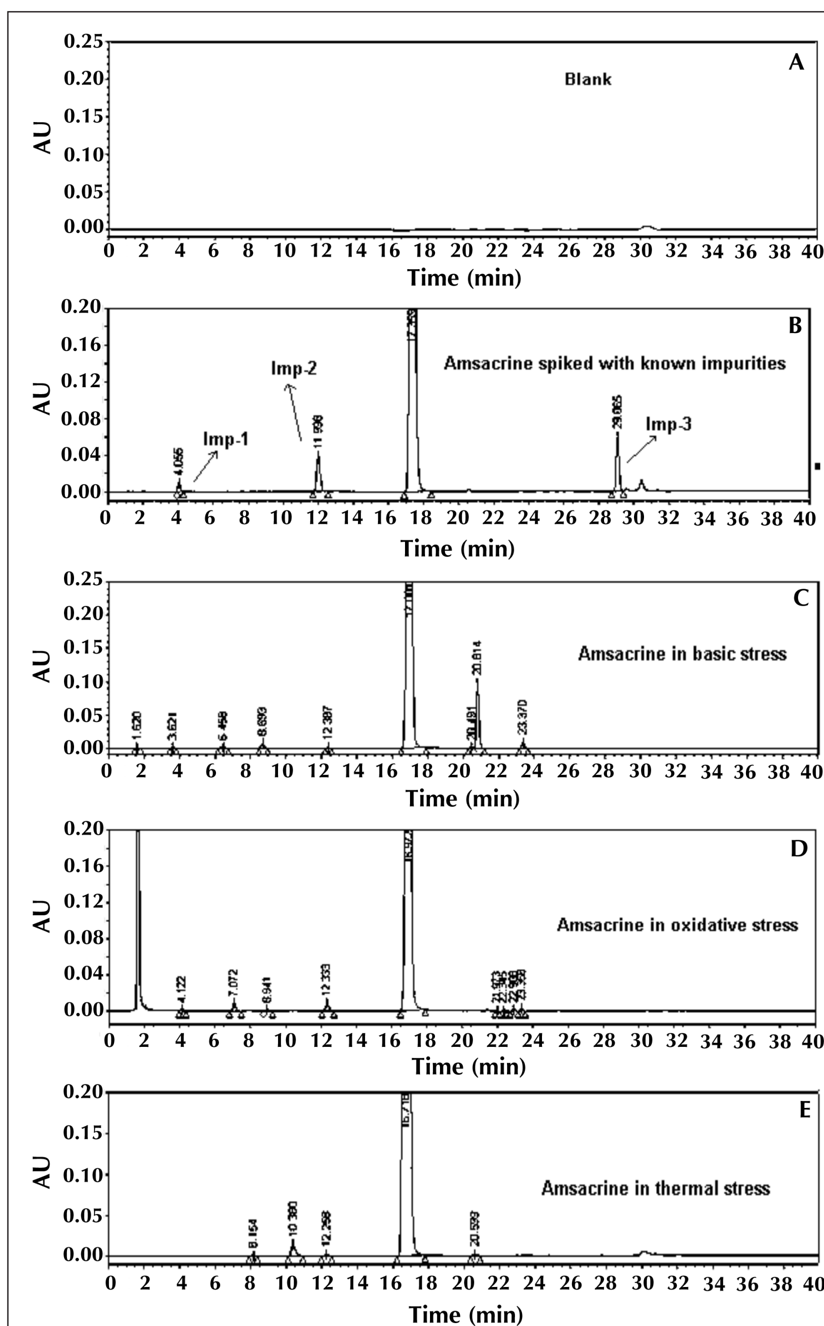


**Figure 2.** Typical HPLC chromatograms of blank (I), Amsacrine spiked with impurities (II), Amsacrine in Basic stress (III), Oxidative stress (IV), and Thermal stress (V).

### Results of forced degradation

Amsacrine was stable under stress conditions such as photolytic stress, acid stress conditions, but significant degradation of the drug substance was observed under basic hydrolysis, which leads to formation of one major degradation product at 1.22 RRT, and oxidative hydrolysis leads to the formation of two major unknown degradation peaks at 0.42 RRT and at 0.74 RRT, along with some small degradation products (Figure 2C and 2D). Slight degradation was observed in thermal stress conditions. From the peak purity test results obtained in the test samples, the purity threshold is greater than purity angle; this confirms that the amsacrine peak is homogeneous and pure in all the stress samples analyzed. The mass balance is a process of adding together the assay value and the levels of degradation products to see how closely these add up to 100% of initial value with due consideration of the margin of analytical error (14). The mass balance of stressed samples was in between close to 99.4% to 99.9% (Table I). The assay of Amsacrine was unaffected by the presence of imp-1, imp-2, and imp-3 and degradation products thus confirms the stability-indicating power of the developed LC method.

**Results of method validation**

*LOD and LOQ*

The LOD of imp-1, imp-2, and imp-3 were 0.05, 0.06 and 0.04 µg/mL respectively (of analyte concentration i.e., 300 µg/mL) for 20 µL injection volume. The LOQ of imp-1, imp-2 and imp-3 were 0.15, 0.19, and 0.12 µg/mL respectively (of analyte concentration. 300 µg/mL) for 20 µL injection volume. Precision study was also carried at the LOQ level by injecting six individual preparations of imp-1, imp-2 and imp-3 and calculated the percentage RSD. The results were presented in Table II.

*Linearity*

Linearity was established by least squares regression analysis of the calibration curve. Calibration curve for the assay method was obtained over the calibration ranges tested (i.e., 150–450 µg/mL) of assay analyte concentration, and the correlation coefficient (r) obtained was greater than 0.9998. Linearity was checked for the assay method over the same concentration range for two consecutive days. The results show that an excellent correlation existed between the peak area and concentration of the analyte. Linear calibration plot for the related substance method was obtained over the calibration ranges tested (i.e., LOQ to 0.6 µg/mL for imp-1, imp-2, and imp-3). The correlation coefficient ($r$) obtained was greater than 0.9988. Linearity was checked for the related substance method over the same concentration range for two consecutive days. The results demonstrate that an excellent correlation existed between the peak area and concentration of imp-1, imp-2, and imp-3. The regression equations were as follows

$y = 188117x + 80.146$, $r = 0.9998$ for amsacrine

$y = 68457x + 30.675$, $r = 0.9997$ for imp-1

$y = 134757x - 127.68$, $r = 0.9988$ for imp-2

$y = 687561x - 246.84$, $r = 0.9989$ for imp-3

**Table II. Results of Precision at LOQ level**

| Preparation | Imp-1 Area | Imp-2 Area | Imp-3 Area |
|---|---|---|---|
| 1 | 14353 | 15076 | 11200 |
| 2 | 13405 | 14307 | 12416 |
| 3 | 13210 | 14834 | 13398 |
| 4 | 12851 | 15234 | 11543 |
| 5 | 12875 | 15500 | 11745 |
| 6 | 11516 | 14008 | 11908 |
| Average | 13035 | 14826.5 | 12305 |
| %RSD | 7.09 | 3.84 | 6.48 |

**Table III. Results of Accuracy for Related Substances and Assay**

| Compound | Level % | Spiked quantity (µg/mL) | Recovered quantity (µg/mL) | % Recovery |
|---|---|---|---|---|
| Amsacrine | 50 | 155 | 154 | 99.4 |
| | 100 | 306 | 308 | 100.7 |
| | 150 | 459 | 458 | 99.8 |
| Imp-1 | 0.05 | 0.149 | 0.147 | 98.7 |
| | 0.10 | 0.305 | 0.307 | 100.7 |
| | 0.15 | 0.454 | 0.435 | 95.8 |
| Imp-2 | 0.05 | 0.151 | 0.153 | 101.3 |
| | 0.10 | 0.301 | 0.295 | 98.0 |
| | 0.15 | 0.447 | 0.427 | 95.5 |
| Imp-2 | 0.05 | 0.152 | 0.144 | 94.7 |
| | 0.10 | 0.304 | 0.299 | 98.4 |
| | 0.15 | 0.468 | 0.458 | 97.9 |

*Precision*

The RSD percentage of assay of amsacrine during assay method precision study was well within 1.0% and the RSD percentage of area of imp-1, imp-2, and imp-3 in related substance method precision study was within 11%. The RSD percentage of assay results obtained in the intermediate precision study was within 1.0%, and the RSD percentage of area of imp-1, imp-2, and imp-3 were within 8%, confirming the good precision of the developed method. The method precision for imp-1, imp-2, and imp-3 at LOQ level was below 10% RSD.

*Accuracy*

The percentage recovery of amsacrine in bulk drug samples ranged from 99.4 to 100.5%. The percentage recovery of imp-1, imp-2, and imp-3 in bulk drugs samples ranged from 93.7 to 102.9%, and the results are show in Table III.

*Robustness*

In all the deliberate varied chromatographic conditions (flow rate, column temperature, pH variation), the resolution between amsacrine and imp-2 as well as with other impurities was not significantly affected; hence the developed HPLC method was robust for the determination of amsacrine in bulk samples. The results are shown in Table IV.

**Application of the developed HPLC method to stability samples and quality monitoring of amsacrine**

Accelerated and long-term stability studies are carried out to establish retest period or a shelf life of drug product (15). Amsacrine samples stored at long-term condition (temp.: 30°C ±

**Table IV. Results of Robustness of the Method**

| Parameter | Modification | Resolution between Imp-2 and Amsacrine | USP tailing factor for Amsacrine | % Recovery for Amsacrine |
|---|---|---|---|---|
| pH | 6.3 | 13.5 | 1.21 | 99.6 |
| | 6.5 | 14.2 | 1.02 | 99.8 |
| | 6.7 | 12.5 | 1.13 | 100.1 |
| Buffer | 15 | 11.5 | 1.3 | 99.8 |
| | 20 | 14.9 | 1.01 | 100.6 |
| | 25 | 13.3 | 1.1 | 100.2 |
| Flow | 0.9 | 15.3 | 1.25 | 99.2 |
| | 1.0 | 14.7 | 1.03 | 100.3 |
| | 1.1 | 12.8 | 0.98 | 99.5 |

**Table VI. Results of Stability Samples (Long Term and Accelerated) of Amsacrine (B. No: ABAM08E02)**

| Storage Condition | Period | Description | Water content by KF | Related substances by HPLC | | | any unknown impurity | Total impurities | % Assay |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Imp-1 | Imp-2 | Imp-3 | | | |
| *Long term condition* | | | | | | | | | |
| Temperature | Initial | orange color crystalline solid | 0.22 | 0.02 | ND | ND | 0.03 | 0.05 | 99.9 |
| 30°C ± 2°C, | 1st month | orange color crystalline solid | 0.27 | 0.02 | ND | ND | 0.03 | 0.04 | 99.8 |
| Relative | 2nd month | orange color crystalline solid | 0.25 | 0.02 | ND | ND | 0.04 | 0.05 | 99.7 |
| humidity | 3rd month | orange color crystalline solid | 0.28 | 0.03 | ND | ND | 0.04 | 0.05 | 100.2 |
| 60 ± 5% | 6th month | orange color crystalline solid | 0.31 | 0.02 | ND | ND | 0.03 | 0.05 | 99.9 |
| *Accelerated condition* | | | | | | | | | |
| Temperature | Initial | orange color crystalline solid | 0.22 | 0.02 | ND | ND | 0.03 | 0.05 | 99.9 |
| 40°C ± 2°C | 1 st month | orange color crystalline solid | 0.29 | 0.02 | ND | ND | 0.04 | 0.06 | 99.7 |
| Relative | 2nd month | orange color crystalline solid | 0.31 | 0.03 | ND | ND | 0.04 | 0.04 | 100.3 |
| humidity | 3rd month | orange color crystalline solid | 0.28 | 0.03 | ND | ND | 0.05 | 0.06 | 100.1 |
| 75±5% | 6th month | orange color crystalline solid | 0.29 | 0.02 | ND | ND | 0.05 | 0.05 | 99.8 |

2°C, relative humidity 60 ± 5%) and accelerated condition (temp.: 40°C ± 2°C, relative humidity 75 ± 5%) were analyzed by using the developed HPLC method for a period of 6 months at different intervals (i.e., initial, 1, 2, 3, and 6 months). Also, the quality of amsacrine was monitored during the production of three batches by using the developed HPLC method. The results (Table V) clearly indicated that the drug was stable under long-term and accelerated conditions, and there were no interference of the impurities for amsacrine, which demonstrates that developed HPLC method was stability-indicating and well applied for drug stability studies as well as for quality monitoring of amsacrine.

## Conclusions

In this paper a simple validated and well-defined specific stability indicating HPLC method for the determination of amsacrine as well as its related substances was described, and the behavior of Amsacrine under various stress conditions was studied and presented. All the degradation products and process impurities were well separated from the drug, amsacrine, which demonstrates that the method is stability indicating. The information presented here in could be very useful for quality monitoring of bulk samples, and also employed to monitor the quality of the drug during stability studies.

## Acknowledgments

The authors wish to thank the management of United States Pharmacopeia-India Private Limited group supporting this work. We would also like to thank colleagues in separation science division of Analytical Research of United States Pharmacopeia-India Private Limited for their cooperation in carrying out this work.

## References

1. Erfa Canada Inc. AMSA PD® injection product monograph. Westmount, Quebec; 16 August 2005.
2. Rose BD editor. Amsacrine. UpToDate 16.1 ed. Waltham, Massachusetts: UpToDate®; 2008.
3. MARTINDALE—The Complete Drug Reference (database on the Internet). Amsacrine. Thomson MICROMEDEX®, 2008. Available at: http://www.micromedex.com/. Accessed 22 May 2008.
4. Cancer Research UK Charity Number 1089464, 61 Lincoln's Inn Fields London WC2A 3PX, www.cancerresearchuk.org.uk.
5. P.P. Brons, J.M. Wessels, P.C. Linssen, C. Haanen, P.A. Speth. *J Chromatogr.* **422:** 175–85 (1987).
6. C.L. Chen, K.K. Thoen, and F.M.J. Uckun. High-performance liquid chromatographic methods for the determination of topoisomerase II inhibitors. *J Chromatogr B Biomed Sci Appl.* **764(1–2):** 81–119 (2001).
7. ICH, Text on validation of analytical procedures, Q2A (R1). International Conference on Harmonization, IFPMA, Geneva.
8. US Food and Drug Administration (2001) Guidance for industry: analytical procedures and methods validation: chemistry, manufacturing, and controls documentation. Draft Guidance. US Food and Drug Administration
9. ICH (1996) Photo stability Testing of New Drug Substances and Products Q1B, International Conference on Harmonization, November 1996, IFPMA, Geneva.
10. Ghulam A. Shabir. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J. Chromatogr. A,* **987:1–2** 57–66 (2003).
11. M. Ribani, C.B.G. Bottoli, C.H. Collins, I.S.F. Jardim, L.F.C. Melo. *QuimNova* **27:** 771. (2004) doi:10.1590/S0100-40422004000500017.
12. Validation of Compendial Methods, The United States Pharmacopeia, (2000) 24th edn, USP 24, Section <1225>
13. Validation of Chromatographic methods; Reviewer guidance, Centre for Drug Evaluation and research (CDER).November 1994, CMC 3.
14. M. Bakshi and S. Singh. Development of validated stability-indicating assay methods—critical review. *J Pharm Biomed Anal* **28:** 1011–1040 (2002). doi:10.1016/S0731-7085(02)00047-X
15. Stability Testing of New Drug Substances and Products Q1A (R2). International Conference on Harmonization, February 2003, IFPMA, Geneva

Manuscript received July 14, 2010;
revision received September 25, 2010.