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ANALYTICAL TECHNIQUES FOR THE ESTIMATION OFAGOMELATINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS: A REVIEW

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ABSTRACT

Agomelatine (N-[2-(7-methoxynaphthalen-1-yl) ethyl] acetamide), its antidepressant efficacy has been verified in the treatment of major depressive disorder (MDD).Agomelatine showed significant benefits over paroxetine due to the complete absence of side effects including the associated sexual side effects that are troublesome with some antidepressants. Agomelatine has also proven to have anxiolytic properties and thus may prove to be very useful in the treatment of anxiety disorders. Literature survey reveals that Agomelatine is estimated individually by uv spectrometry, RP-HPLC, LC-MS/MS , Stability indicating determination by HPLC-UV, Stability Under different stress Condition by HPLC-Fluoresence and TLC.

Keywords: Agomelatine, RP-HPLC, LC-MS/MS.UV Spectrometric, HPLC-UV, HPLC, Fluoresence and TLC.

INTRODUCTION

Agomelatine acts as a melatonergic receptor (MT1 /MT2) agonist and serotonergic receptor (5-HT2C) antagonist. Binding studies indicate that it has no effect on monoamine uptake and no affinity for α, β adrenergic, histaminergic, cholinergic, dopaminergic, and benzodiazepine receptors [1-3]. Agomelatine showed significant benefits over paroxetine due to the complete absence of side effects including the associated sexual effect that are troublesome with some antidepressant. Because of its action upon the melatonine receptors, agomelatine shows a marked improvement on sleep. Agomelatine has also proven to have anxiolytic properties and thus may prove to be very useful in the treatment of anxiety disorders [2].

Electrospray Ion Trap Mass Spectrometry

Electrospray Ion Source is the key to using MS for solutions is the ability to transfer your analytes into the vacuum of the mass spectrometer as ionic species. This process is handled by an electrospray ion source in our instrument. Positive ion electrospray: Electrospray ion sources are soft ionization sources that are they produce mostly protonated molecular ions, MH+. For small molecules, electrospray produces only one peak, the MH+ peak at mass M+1.You may be familiar with electron impact ionization, which is the normal ionization mode for GC/MS. Electron ionization, EI, is caused by a beam of 70 eV electrons in thesource. EI provides enough energy to fragment the molecular ion, so that many fragment ions occur in the spectrum. EI spectra are often quite complicated. Electrospray spectra are much easier to interpret than EI spectra and the molecular weight of your compound is easily determined. The ions in an electrospray source can be formed in your original solution. For example, quaternary amines, R4N+, are by their nature already are ions. Ions can be formed from basic compounds by protonation by added acids. The proton transfer can occur in your solution or in the droplets produced by the electrospray source. M + H+ -> MH+ If your compound has several basic sites, like a small peptide with several amine sidechains, multiply charged ions may also be formed: M + 2 H+ -> MH2 2+ and M + 3 H+ ->

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MH3 3+ etc.Under these circumstances, several ions will appear in the spectrum, the MH+, MH2 2+,and MH3 3+. The MS determines the m/z value, that is the mass divided by the charge. If we take an example of a compound with mass 300 Da, the spectrum will contain ions at MH+ = 300+1=301 Da, MH2 2+= (300+2)/2=151 Da, and MH3 3+= (300+3)/3=101 Da.Proteins can often produce very high charge states with z ~ 40 or more. Small molecules usually show only one predominant charge state. From this discussion, it is easily seen that pH control for the sample solution can have a strong effect on the ionization efficiency and the distribution of the charge states for your analyte ions. As a consequence, sample solutions for electrospray MS usually are buffered or have added acids to enhance and control the formation of ions. Amines are obvious examples of types of compounds that are easily protonated.However, even alcohols can be protonated in the strongly acidic environment that occurs as the droplets of solution evaporate in the ion source. The spray tip of the ion source must carry the electrospray current, so it acts as the anode of an electrochemical cell. Therefore, if your molecule can't be easily protonated, ions may still be formed by electrochemical processes in the spray tip. Through direct protonation or electrochemical oxidation, most types of compounds can be analyzed. Nonreactive hydrocarbons are not detectable by electrospray ionzation. Negative ion electrospray: Acidic analytes can also be detected using negative ion electrospray.MH -> H+ + M the detected ion is the M- ion, at mass M-1. Just like positive ion electrospray, negative ion electrospray is a soft ionization technique that can produce multiply charged ions. For example, DNA readily forms polyanions with a range of z values. Negative ion electrospray is commonly, but not always, run from basic or neutral solutions to increase the formation of negative ions. So just like positive ion electrospray, buffered sample solutions are common. Buffer Additives: Because of the need to control ion formation, buffers are very common in direct infusion and LC/MS. However, standard buffers like Tris, HEPES, and phosphate buffers are non-volatile and can clog the MS inlet capillary. Therefore, nonvolatile additives are necessary. The most common buffer components are formic acid, trifluoroacetic acid, acetic acid, ammonium formate, ammonium acetate, and heptafluorobutyric acid (in that order). For more basic buffers, 10-20 mMtetraethylammoniumformate or bicarbonate is common. Often compromises in buffering capacity are made by choosing one of these volatile buffer components. Trifluoroacetic acid can suppress ion formation in electrospray, so its concentration is usually keptionization, EI, produces fragment rich spectra. The fragment ions are useful to help determine the structure of the compound. On the other hand, in EI some classes of compounds don't produce intense parent peaks, so the molecular weight is difficult to determine. While the ease of molecular weight determination is a strength for electrospray, the lack of structural information from fragment ions can be a draw back. MS/MS techniques can solve this problem. In MS/MS analysis, the MH+ ions formed from the electrospray source are fragmented by adding extra collisional energy. Our MS is based on an ion trap analyzer. In ion traps, ions can be held for long time periods, giving an easy opportunity to fragment the parent MH+ ions. The trap is always filled with about 1 Torr of helium gas. A small radiofrequency field can be applied to the trap to cause the ions to move faster. The parent ions collide with the helium background gas causing fragmentation. After adding this collisional energy, the resultant ions are scanned in the normal way to determine their m/z. This process is called collision-induced dissociation, CID. CID MS/MS spectra are very similar to EI spectra and can be interpreted in the same way. MS/MS spectra can be acquired manually by selecting the mass of the parent ion to be fragmented and the amount of collisional energy. MS/MS can also be done automatically. In Auto MS/MS mode, the computer determines the mass of the most intense parent ions and subjects those ions to MS/MS. The user can choose the maximum number of parent ions to be fragmented. The important parameter for MS/MS based analysis in LC/MS, is that MS/MS takes extra time. If the eluting peaks are too narrow, then there won't be time for MS/MS analysis. As a consequence, some compromise in resolution and retention time may be necessary to do auto MS/MS analysis. Strangely, this means that the best efficiency isn't always best for MS/MS detection, which is a strange circumstance for most chromatographers.

MS/MS analysis is particularly useful for biopolymers. Proteins, peptides, and oligonucleotides can be sequenced using MS/MS. The auto MS/MS analysis of proteolytic digests of proteins is one of the two MS techniques that have spawned the new field of proteomics. (The other MS technique is MALDI.) Sample Introduction:Electrospray mass spectrometers can be used in two modes. In direct infusion mode, a dilute solution of the analyte is pumped into the source. No separation takes place and all the components of the sample give peaks in the mass spectrum simultaneously. For simple mixtures this is fine, but for complex samples direct infusion produces a forest of peaks that are difficult to distinguish from the noise. For complex samples, an HPLC is commonly attached to the source. The source then ionizes the analytes separately as they elute from an HPLC column. The samplefor LC/MS is usually more concentrated than for direct infusion to compensate for the dilution factor of the HPLC separation. Typical volumes for direct infusion are 20-200 ? L, while HPLC requires 50 nL-5 ? L of more concentrated sample.LC/MS is the most common detector for HPLC is a UV detector. However, a mass spectrometer provides a means of identifying the components in different peaks. MS is a very powerful tool, but your HPLC method and sample preparation must be

carefully designed to achieve good detection limits. With careful experimental design, electrospray MS can be as sensitive as or even more sensitive than a UV detector. pH control for the HPLC eluant can have a strong effect on the ionization efficiency and the distribution of the charge states for your analyte ions. As a consequence, HPLC eluants for electrospray MS usually are buffered or have added acids to enhance and control the formation of ions. Volatile additives must be used to avoid clogging the transfer capillary. Most common HPLC packings shouldn't be used at pH7, so care must be exercised when designing your analysis for negative ion mode. Flow rates: The electrospray interface can use two different spray needles. The standard interface uses a stainless steel needle. This needle has a relatively large bore, and therefore a relatively large hold-up volume. This large volume causes extra band broadening when the interface is used with capillary HPLC. The flow rate range for the standard source is roughly 4-1000 ? L/min. 10-100 ? L/min is a good starting range. In HPLC, the volume of the tubing, connections, and detector cells is called the extracolumn volume. In other words, the extra-column volume is any volume that does not include the column. Extra-column volume is a source of band broadening. To decrease the extra-column volume, an alternate spray needle is available. This needle is made from 50 ?m ID fused silica tubing. The tip of the needle is specially ground into a conical shape to help increase the efficiency of the source for low flow rates. These ―taper-tips‖ are made by the New Objective Company in Cambridge, MA. One disadvantage of these spray needles is that the tip is very fragile. The small ID also increases the backpressure of the interface. For this reason, the usable flow rate maximum is less than the stainless steel needle. The back pressure from the source should be kept low to avoid rupturing the diode array detector cell. If the source is fitted with the taper-tip fused silica tip, the maximum flow rate is decreased to 50 ? L/min. Using greater than 50 ? L/min will cause a costly repair and long down time. The normal flow rate for 1 mm columns is 50? L/min. You need to be aware of the tip that is installed in the source, to determine a good operating flow rate. If you are unsure, assume thatthe taper-tip is being used. Also when cleaning the source you should be careful to avoid touching the end of the spray needle.

Instrumentation of mass spectrometric detection

Mass spectrometry is a particularly powerful scientific technique because it can be successfully applied even if you have only a tiny quantity available for analysis—as little as 10-12 g, 10-15 moles for a compound of mass 1000 Daltons (Da). Compounds can be identified through mass spectrometry at very low concentrations (one part in 1012) in chemically complex mixtures. The basic mass spectrometry processes of instrumentation are consisted of [1], introduction of sample; a sample which can be a solid, liquid, or vapor is loaded onto a mass spectrometry device and is vaporized [2], ionization; sample components are ionized by one of several available methods to create ions [3], analyzer sorting; the ions are sorted in 445 an analyzer according to their m/z ratios through the use of electromagnetic fields [4], detector; the ions then pass through a detector where the ion flux is converted into a proportional electrical current and [5], date conversion; the magnitude of the ion/electrical signals is converted into a mass spectrum (Watson & Sparkman , 2007). MS instruments consist of three modules: an ion source, which can convert gas phase sample molecules into ions (or, in the case of ESI, move ions that exist in solution into the gas phase); a mass analyzer, which sorts the ions by their masses by applying electromagnetic fields; and a detector, which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present. The technique has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds**.**

ANALYTICAL METHODOLIGIES

Sridhar Thota*et al.,* Agomelatine is a new melatonergic antidepressant with a unique pharmacological action. A stability-indicating RP-HPLC method was developed and validated for the determination of agomelatine in active pharmaceutical ingredient using enable C18 column (150×4.6mm, 5µm) in isocratic mode. The mobile phase consisted of acetonitrile: methanol: water (55:25:20, v/v/v) with a flow rate of 1.0 ml/min (UV detection- 230nm). The Retention time was found to be 4.2 min. Linearity was observed over the concentration range of 19 ng/ml to 60µg/ml and the correlation coefficient R2value was found to be 0.9988. The method is accurate and recovery was found to be in the range of 98-100.7%. The limit of detection of agomelatine was found to be 4ng/ml and limit of quantitation was found to be 15 ng/ml. Agomelatine was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Agomelatine is more sensitive to acidic and oxidative degradation. The method was validated according to ICH guidelines [1].

Vineela P *et al.,*Agomelatine is a new melatonergic antidepressant with a unique pharmacological action. A stability indicating RP-HPLC method was developed and validated for the determination of agomelatine in active pharmaceutical ingredient using enable thermo hypersil C18 column (250×4.6mm, 5µm) in isocratic mode. The mobile phase consisted of phosphate

buffer: methanol (60:40, v/v) with a flow rate of 1.0 ml/min (PDA detection- 232nm).The Retention time was found to be 3.3 min. Linearity was observed over the concentration range of 25 µg/ml to 75µg/ml and the correlation coefficient R2value was found to be 0.999. The method is accurate and recovery was found to be in the range of 98.91-99.18%. The limit of detection of agomelatine was found to be 2.8µg/ml and limit of quantitation was found to be 9.4µg/ml. Agomelatine was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Agomelatine is more sensitive to heat and oxidative degradation. The method was validated according to ICH guidelines [2].

Joshi Hitendra Set al., A simple, precise and stability-indicating high performance thin chromatographic method for analysis of Agomelatine in bulk drug and in a tablet formulation has been developed and validated. Aluminum foil TLC plates pre-coated with silica gel 60F were used as stationary phase Dichloro methane and methanol in the ratio of (95:5v/v) were used as mobile phase. A compact band (Rf 0.52±0.002) was obtained for Agomelatine. Densitometric analysis was performed in absorbance mode at 230 nm. Linear regression analysis revealed a good linear relationship (r2= 0.9982) between peak area and concentration in the range of 0.2-0.8 µg/spot. The precision (relative standard deviation: RSD) among a six sample preparation was 0.94% and 0.93%. The accuracy (recovery) was validated for specificity, linearity, precision, recovery and robustness. The limits of detection and quantization were also determined. Statistical analysis proved the method enables repeatable, selective and accurate for analysis of the drug. It can be used for identification and quantitative analysis of Agomelatine in bulkdrug and in tablet formulation [3].

M. Vijaya Lakshmi *et al.*,The aim of the present work was to develop and validate a simple, efficient, economical RP-HPLC method for the estimation of Agomelatine (AMT) in bulk and dosage forms. A Phenomenax C18 column (250 x 4.6mm; 5µm) with mobile phase containing water: methanol (20:80% v/v) at a flow rate of 1mL/min was used and quantification was carried at 230nm. The retention time of agomelatine was 5.09 min and showed a good linearity in the concentration range of 10-50µg/mL for agomelatine with a correlation coefficient of 0.998. Validation parameters fulfilled regulatory requirements in all cases. The percent recoveries were found to be 100.64 with RSD less than 2. The proposed HPLC method was validated as per ICH guidelines and successfully applied for the estimation of agomelatine in bulk and dosage forms [4].

K. Y. Janga*et al.*,Purpose**:**To explore a new validated Reverse phase High performance liquid chromatographic method for agomelatine, a melatonergicantidepressant, and to estimate entrapment efficiency and drug content in proliposome powder formulation. Methods: Central composite design (CCD) with surface response curves employed to obtain suitable liquid chromatographic medium.The most selective mobile phase composition was deduced from the responses of CCD. Calibration was attained for bestsuitable chromatographic conditions and validated with respect to linearity, range, specificity, accuracy, precision,robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ). Agomelatine loaded proliposomal powder with equimolar fractions of hydrogenated soya phosphatidyl choline and cholesterol was acquired by Film depositiontechnique. Quantification of percentage drug content and entrapment efficiency within formulation was executed inoptimized conditions at 230 nm in UV detector allied with Shimadzu HPLC system (Japan).Results Responses accrued from CCD evinced optimized mobile phase composition of acetonitrile : water (55: 45) (v/v) at 250C forcomplete elution of drug in Phenomenex stainless steel C18 column (250 x 4.6mm,5µm) at a flow rate of 1mL/min withretention time of 5.81± 0.26 minutes. A good linear relationship with the mean (n=3) correlation coefficient value, r2 =0.999±0.001 was exuded when calibration plots build in the concentration range of 0.25 - 8µg /ml by means of thischromatographic conditions. Adequate intraday and interday precision and accuracy were observed over a linear range. LODand LOQ values were found to be 0.066 & 0.20 µg/ml respectively. A free flowing and stable proliposome powder formulation was procured. Percentage Drug content of 98.2 ±2.16 and 90% of entrapment efficiency of drug in liposome waselucidated.simple, linear, reliable, specific, robust, accurate and precise validated method developed with the aid of CentralComposite Design confides it as a potential tool to explicate the quantification of agomelatine in proliposome powder formulation [5].

A simple and highly sensitive stability-indicating HPLC method was developed and validated for the determination of the new antidepressant agent, agomelatine (AGM). Separation of AGM from its stress-induced degradation products was achieved on a BDS Hypersil phenyl column (250 mm × 4.6 mm i.d., 5 µm particle size) using methanol-0.05 M phosphate buffer of pH 2.5 (35: 65, v/v) as a mobile phase with fluorescence detection at 230/370 nm. Naproxen was used as an internal standard. The method satisfied all the validation requirements, as evidenced by good linearity (correlation coefficient of 0.9999, over the concentration range 0.4-40.0 ng/mL), accuracy (recovery average 99.55 ± 0.90%), precision (intra-day RSD 0.54-1.35% and inter-day RSD 0.93-1.26%), robustness and specificity. The stability of AGM was investigated under different ICH recommended stress conditions including acidic, alkaline, neutral, oxidative and photolytic. AGM was found to be labile to acidic and alkaline degradation and a kinetic study was conducted to explore its degradation behavior. First-order degradation rate constants and half-life times were calculated in each

case. The proposed method was applied for the determination of AGM in tablets and spiked human plasma with mean percentage recoveries of 99.87 ± 0.31 (n = 3) and 102.09 ± 5.01 (n = 5), respectively. Hence, the proposed method was successfully applied for the determination of AGM in human volunteer plasma. The results were compared statistically with those obtained by a comparison HPLC method revealing no significant differences between the two methods regarding accuracy and precision [6].

Satish R *et al.,* An analytical method based on liquid liquid extraction has been developed and validated for analysis of agomelatine in human plasma. Fluoxetine was used as an internal standard for agomelatine. A Betasil C18 (4.0100mm, 5mm) column provided chromatographic separation of analytes followed by detection with mass spectrometry. The method involves simple isocratic chromatographic conditions and mass spectrometric detection in the positive ionization mode using an API-4000 system. The proposed method has been validated with linear range of 0.050–8.000ng/ml for agomelatine. The intra-run and inter-run precision values are within 12.12% and 9.01%, respectively, for agomelatine at the lower limit of quantification level. The overall recovery for agomelatine and fluoxetine was 67.10% and 72.96%, respectively. This validated method was used successfully for analysis of plasma samples from a pharmacokinetic study. Copyright © 2012 John Wiley & Sons, Ltd. [7].

NR Akmar*et al.,* Agomelatine is a new melatonergic antidepressant with a unique pharmacological action. A UV spectroscopy and stability-indicating RP-HPLC method was developed and validated for the determination of Agomelatine in active pharmaceutical ingredient and tablet dosage form. In UV Spectroscopic method methanol was used as solvent and ?max was found at 230nm. Beer's law was observed in the concentration range of 0.5- 3µg/ml (R2 = 0.997). LOD and LOQ were 0.04798 and 0.145395µg/ml respectively. The method was validated for several parameters like accuracy, precision. The values of % RSD and % recovery were found to be satisfactory. RP-HPLC method was developed using Thermo BDS Hypersil C18 column (250 × 4.6 mm, 5µm) in isocratic mode. The mobile phase consisted of Acetonitrile: 15mM Phosphate buffer pH 5 (40:60 v/v) with a flow rate of 1.0 ml/min (UV detection- 230nm). The Retention time was found to be 6.9 min. Linearity was observed over the concentration range of 5-30µg/ml (R2 = 0.996). The method is accurate and recovery was found to be in the range of 99.89-100.19%. The limit of detection of Agomelatine was found to be 0.1218µg/ml and limit of quantitation was found to be 0.3691µg/ml. Agomelatine was subjected to stress conditions including acidic, alkaline, neutral, oxidation and thermal degradation. Agomelatine is more sensitive to acidic, basic and oxidative degradation. These methods were validated according to ICH guidelines [8]

Liu Y*et al.,* Seven impurities in agomelatine drug substance were determined by a newly developed RP-HPLC method. Structures of potential impurities were confirmed by NMR and IR analysis. Efficient chromatographic separation was achieved on Hypersil BDS C18 column (250 mm × 4.6 mm, 5 µm) in gradient mode by using a binary mixture of potassium dihydrogen phosphate (15 mM, pH adjusted to 3.0) and acetonitrile at a flow rate of 1.0 ml/min. A photodiode array detector set at 230 nm was used for detection. Forced degradation studies showed that the proposed method was specific, and agomelatine was found to be susceptible to acidic and alkaline conditions.

CONCLUSION

The method was validated according to ICH guidelines with respect to specificity, sensitivity, precision, linearity, accuracy, robustness and system suitability. Detection limit of impurities was in the range of 0.0008-0.0047%. Regression analysis showed correlation coefficient value greater than 0.999 for agomelatine and its seven impurities. Accuracy of the method was established based on the recovery obtained between 94.4% and 106.7% for all impurities. The validation results demonstrated that the developed method was suitable for the quantitative determination of potential impurities in agomelatine. A possible mechanism for the formation of impurities was proposed [9].

Fig 1. Agomelatine

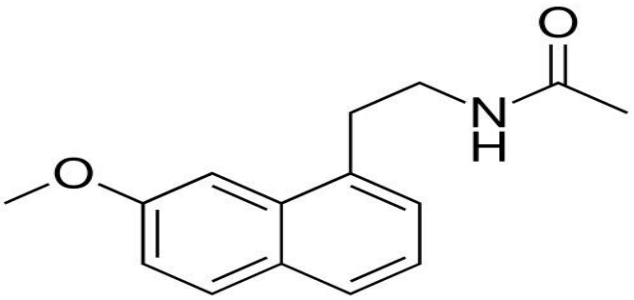


Fig 2. Electron Spray Ion Source

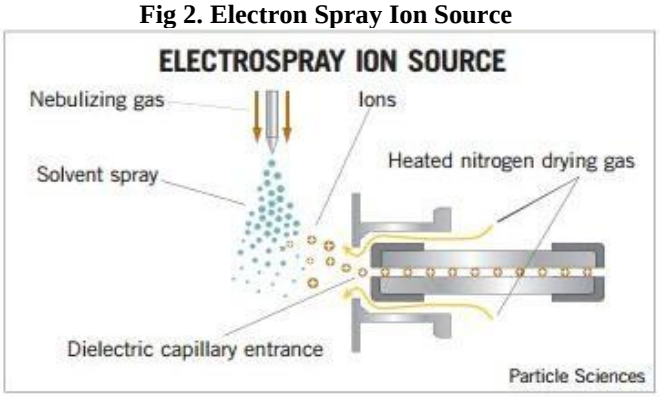
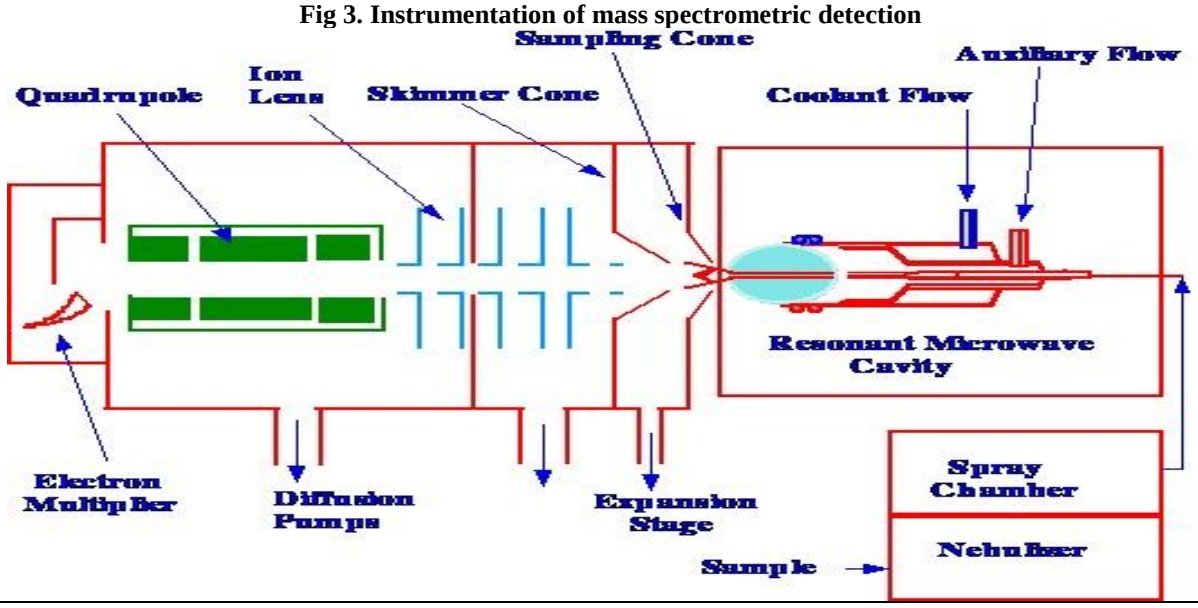


Fig 3. Instrumentation of mass spectrometric detection



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