

- [54] **METHOD OF COUPLING THIN LAYER CHROMATOGRAPH WITH MASS SPECTROMETER**
- [75] Inventors: **Robert M. Parkhurst**, Redwood City; **James H. McReynolds**, Palo Alto, both of Calif.
- [73] Assignee: **Stanford Research Institute**, Menlo Park, Calif.
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- [58] Field of Search 73/23.1, 61.1 C, 422 GC; 250/288, 425; 210/31 C, 198

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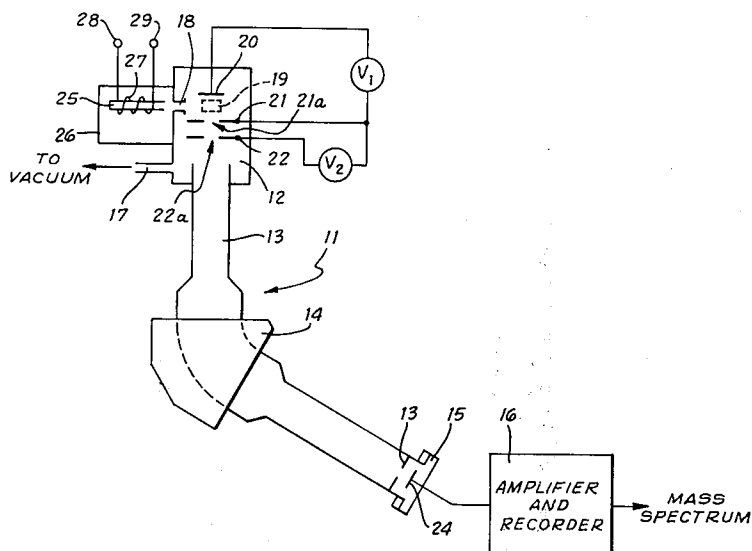
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[57] **ABSTRACT**

A method of analyzing a mixture of chemical substance in which a thin layer chromatograph is formed by placing a quantity of the mixture or a solution of the mixture at one end of a chromatographic medium of a sorptive material and through differential migration of the various chemical substances in the mixture separated zones of absorbed chemical substances are created on the chromatographic medium. The chromatographic medium itself is then placed in a vacuum immediately adjacent the ion source of a mass spectrometer. The various zones of absorbed chemical substances are then heated one at a time. As each zone is heated the absorbed chemical substance sublimates directly into the ion source of the mass spectrometer, and a mass spectrograph of that particular zone of chemical substance is formed.

6 Claims, 6 Drawing Figures

- [56] **References Cited**
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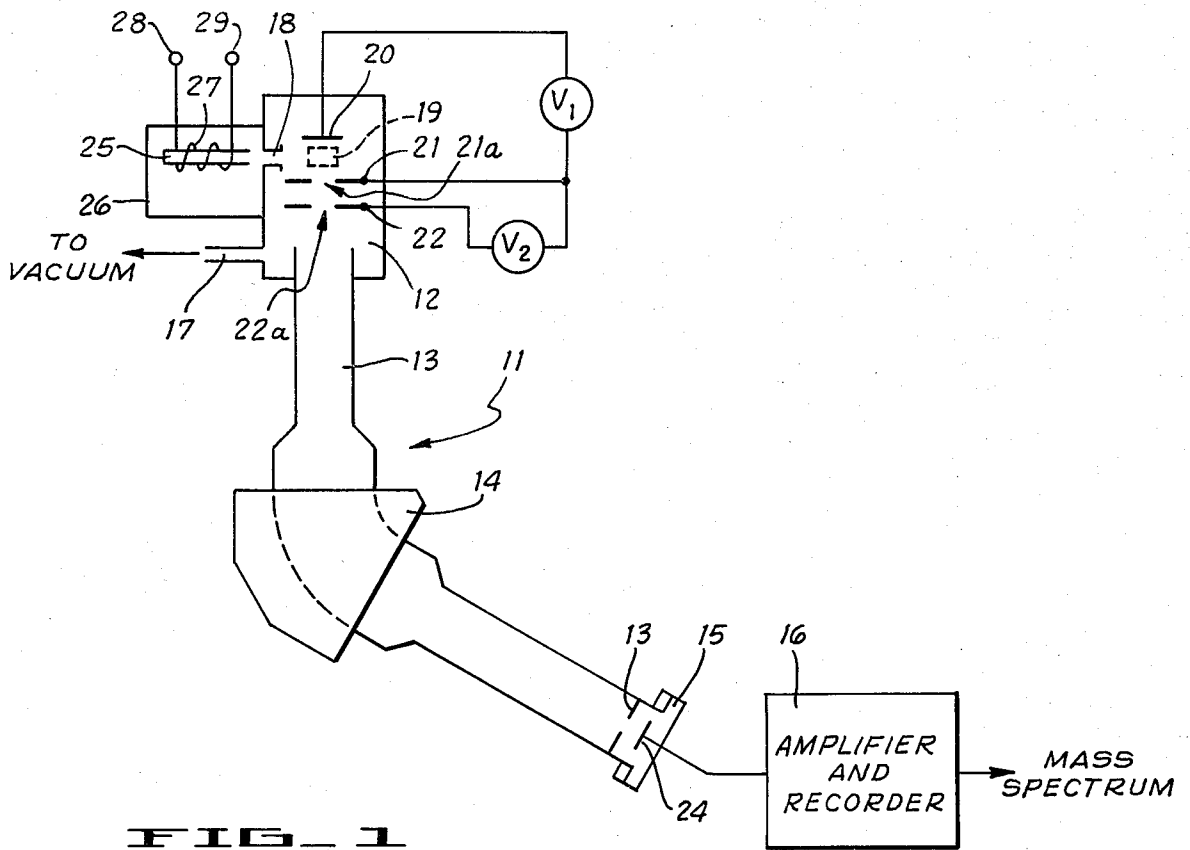


FIG. 1

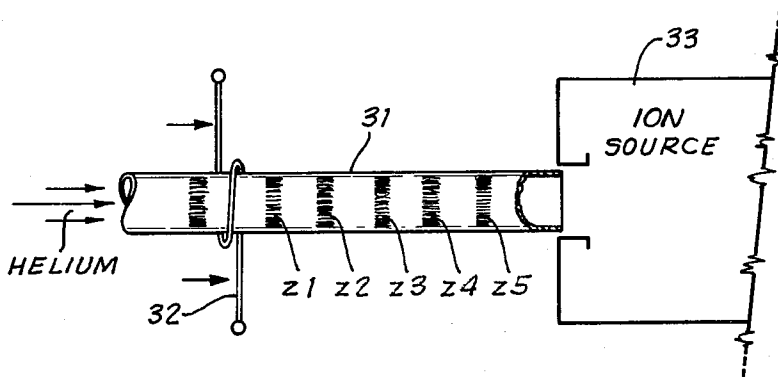


FIG. 2

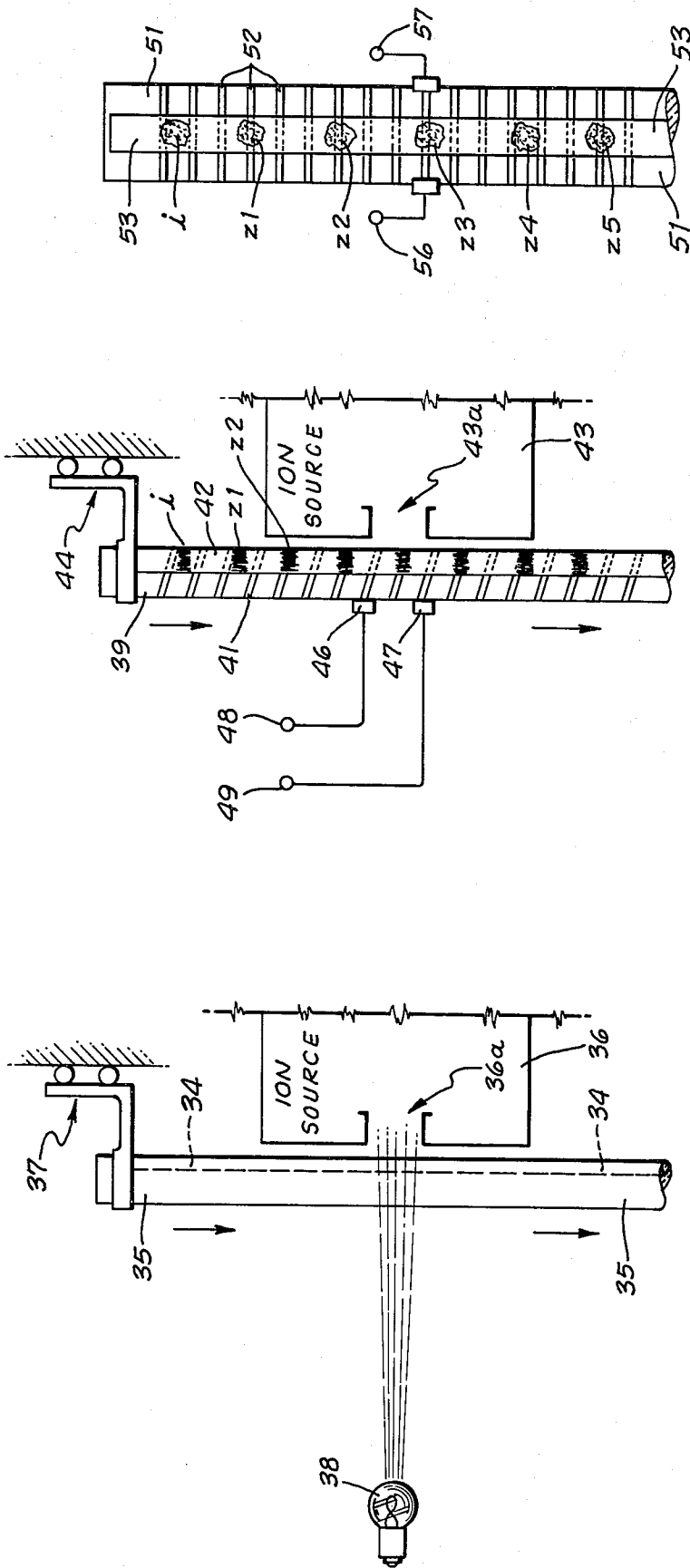


FIG. 5

FIG. 4

FIG. 3

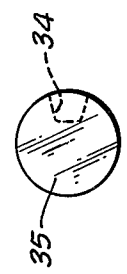


FIG. 3A

METHOD OF COUPLING THIN LAYER CHROMATOGRAPH WITH MASS SPECTROMETER

BACKGROUND OF THE INVENTION

This invention pertains to a method for analyzing mixtures of chemical substances and in particular pertains to such a method involving a combination of thin layer chromatography and mass spectroscopy.

In the field of analysis of complicated chemical mixtures, there have been methods developed in which different structure-finding techniques have been coupled together. As an example, there are described in an article entitled "TLC In Direct Coupling with GC and MS", Kaiser, R., Chemistry in Britain, 5(No. 2) 54 (1969), various proposed methods for coupling together analytical techniques. One method involves the coupling together of gas chromatography with a mass spectrometer. The use of gas chromatography requires that the compounds to be separated be volatile at a temperature suitable for gas chromatography. Certain complicated organic mixtures such as natural products, drug metabolites and clinical samples are not sufficiently volatile to be separated by gas chromatography. Furthermore, the use of gas chromatography directly coupled to a mass spectrometer requires the use of gas separators (helium separates, for example) to remove the large amounts of carrier gas. The article referred to above also has proposed a combination of thin layer chromatography with gas chromatography and a mass spectrometer. In this arrangement a thin layer chromatography is formed on a plate. A flame torch is utilized on the back of the thin layer chromatograph plate which is not in close proximity to the ion source to successively evaporate the zones thereon. These zones are successively evaporated at atmospheric pressure into a nitrogen or helium gas stream for flow into a mass spectrometer. Again, this technique requires that the compounds forming the various zones have a rather high vapor pressure since they must evaporate into the gas stream.

These techniques in the prior art have been somewhat deficient in not being applicable to complex non-volatile mixtures which require analytical determination.

OBJECTS AND SUMMARY OF THE INVENTION

Accordingly, it is an object of this invention to provide a method for analyzing complex non-volatile mixtures.

It is another object of this invention to provide a method for analyzing complex mixtures which directly couples thin layer chromatography with mass spectroscopy.

Briefly, in accordance with one embodiment of the invention, a thin layer chromatograph of the mixture to be analyzed is formed wherein differential migration of substances in the mixture forms physically separate absorbed zones of the substances on the chromatographic medium. The thus formed thin layer chromatograph is then placed in a vacuum in close proximity to an ion source of a mass spectrometer. The physically separated zones of the thin layer chromatograph are then heated, one at a time, to sublime the substance in a zone directly into the ion source of the mass spectrometer. A mass spectrograph is then formed of each of the substances.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagrammatic illustration of one means of practising the invention by directly combining a thin layer chromatograph adjacent to the ion source of a mass spectrometer.

FIG. 2 is a schematic illustration of one embodiment of a thin layer chromatograph in accordance with the invention adjacent to an ion source of a mass spectrometer.

FIG. 3 is similar to FIG. 2 and shows another embodiment of a thin layer chromatograph.

FIG. 3a is an end view of the thin layer chromatograph of FIG. 3.

FIGS. 4 and 5 are illustrations of still other embodiments of thin layer chromatographs for practising the method of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The basis of this invention is a method of coupling thin layer chromatography with a mass spectrometer detection system. The method is generally applicable to analyzing virtually any kind of mixtures which can be chromatographically separated and is especially suitable for analyzing complicated organic mixtures such as natural products, drug metabolites and clinical samples which are not sufficiently volatile to be analyzed by gas chromatography - mass spectrometer techniques.

Referring now to FIG. 1, there is shown a diagrammatic illustration of a typical arrangement for practising the method of the invention. A mass spectrometer 11 of any of the conventional types is suitable for use in practising the invention. The specific arrangement (magnetic sector instrument) shown in FIG. 1 is intended only by way of an example. Other mass analyzers such as the quadrupole or time of flight are equally applicable. The mass spectrometer 11 includes an ion source portion 12, a tube 13, magnetic structure 14, collector portion 15 and suitable electronics 16. The ion source portion 12 and the tube 13, as well as the collector portion 15, are all in a vacuum with an appropriate vacuum connection 17 to the vacuum pump (not shown). The ion source portion 12 includes an inlet 18 into which molecules of a sample to be analyzed are introduced. There is also provided a suitable electrode structure 19 wherein a stream of electrons generated by a filament in the conventional manner strikes the molecules of the sample causing ionization and fragmentation in a conventional manner. A pair of electrodes 20 and 21 are provided with a voltage source V_1 maintaining a small potential of a few volts for directing the ions through an aperture 21a in the electrode 21. An additional electrode 22 is provided having an aperture 22a therein and a relatively large potential is maintained between the electrodes 21 and 22 by a voltage source V_2 , on the order of several thousand volts. The ions and fragmented ions are thus accelerated through aperture 22a through the spectrometer 11 and those ions which pass through an exit aperture 23 are collected by an electrode 24 which is connected to suitable electronics for generating the mass spectrum of the sample, all in a conventional manner.

In accordance with the invention the sample molecules are introduced into the ion source portion 12 of the mass spectrometer from a thin layer chromato-

graph in very close proximity to the ion source portion and in a vacuum. The physically separated zones of the thin layer chromatograph are successively heated, one at a time, as described more fully hereinafter and the substances forming the various zones of the thin layer chromatograph need only sublime a short distance in a vacuum directly into the ion source of the mass spectrometer. Thus substances having a very low vapor pressure can be easily introduced from a thin layer chromatograph into the mass spectrometer by a direct coupling of the two.

Referring again to FIG. 1, the thin layer chromatograph 25 (examples of which are discussed hereinafter) is suitably mounted in a vacuum enclosure 26 which communicates directly with the ion source through the inlet 18. Means are provided to heat the various zones of the thin layer chromatograph 25 one at a time to cause the substances to sublime into the ion source. Various techniques are suitable for applying heat to the thin layer chromatograph as discussed hereinafter. One suitable method is electrical heating which has been diagrammatically illustrated in FIG. 1 as a heating coil 27 surrounding the thin layer chromatograph 25 and having electrical connections 28 and 29 to which a voltage source can be connected.

The remaining part of the description will be devoted to a description of various examples of methods of preparing suitable thin layer chromatographs in accordance with the invention and introducing the separated substances from the chromatograph into the ion source of the mass spectrometer.

Turning now to a consideration of FIG. 2, there is shown an example of practising the method of this invention in accordance with one embodiment. In this embodiment there is provided means such as a glass tube 31 on the inside of which there is formed a layer of a sorbent suitable for use in forming a thin layer chromatograph. This may be a silica-gel slurry, for example, which is allowed to dry. The chromatograph is run in the conventional manner by depositing a sample of the mixture to be analyzed at one end of the tube (indicated by region *i* in FIG. 1). A mobile phase or solvent is passed through the inside of the tube 31 over the region *i* through the inside of the tube. Owing to their selective sorption, the constituents of the mixture migrate with the solvent or mobile phase at different rates and separate from one another as a series of zones z_1 , z_2 , z_3 , etc., is absorbed in the silica-gel and physically separated from one another along the length of the glass tube. Then the thus formed thin layer chromatograph is placed in a vacuum enclosure communicating with and in close proximity with the ion source of the mass spectrometer. Next, in accordance with the principles of the invention heat is applied to the series of zones z_1 , z_2 , etc., one at a time, to cause the absorbed substances in each of the zones to sublime into the ion source of the mass spectrometer. As illustrated in FIG. 1, this can be conveniently done by means of a heating coil 32 which is selectively moved along the outside of the tube 31 from zone to zone. As illustrated in FIG. 2, a flow of a gas such as helium may be introduced into the tube 31 to carry the sublimed substances into the ion source 33 of the mass spectrometer. Mass spectrographs are run in a conventional manner of each of the individual substances which are sublimed from the zones z_1 , z_2 , etc., into the ion source of the mass spectrometer.

Turning now to a consideration of FIGS. 3 and 3a, there is shown a schematic illustration of another embodiment of practising the method of this invention. In accordance with this embodiment a silica-gel or other suitable chromatograph medium is deposited in a groove 34 formed in a glass rod 35. The chromatograph is then run in a customary manner such as discussed before in connection with FIG. 2, with differential migration of the various substances in the sample producing a series of physically separated absorbed zones of substances in the silica-gel medium along the length of the rod 35. The rod 35 with the separated zones of absorbed substances along its length is then placed in a vacuum in close proximity to the ion source 36 of a mass spectrometer. Suitable means such as the mechanical advancing mechanism 37 are provided extending within the vacuum enclosure for selectively advancing the glass rod 35 past the inlet 36a to the ion source so as to successively position each of the zones of absorbed substances adjacent the inlet 36a. In accordance with the principles of the invention, as each of the zones of absorbed substances is positioned in front of the inlet 36a, heat is applied thereto. In the embodiment of FIG. 3 the heat is applied by means of a light source 38 focusing a light beam through the glass rod onto the silica-gel having the absorbed substance thereon. In the embodiment of FIG. 3 the light source can be an ordinary focused source of infrared or visible light or can be, for example, a laser. Each of the absorbed substances is in this manner evaporated from the silica-gel on the glass rod into the ion source of the mass spectrometer. As each substance is thus evaporated, a mass spectrograph is run in the conventional manner.

Turning now to a consideration of FIG. 4, there is shown another form of practising the method of the invention. In the arrangement shown in FIG. 4 there is provided a glass rod 39 which has a continuous spiral of metal 41 formed thereon. The metal 41 is preferably one having a relatively high resistance so that it can function as a resistance heater, as hereinafter described. Thereafter, one half of the glass rod 39 is coated with a suitable chromatograph medium, such as a silica-gel 42 for example. A mixture *i* which is to be analyzed is placed at one end of the glass rod on the silica-gel and the chromatograph is run in a conventional manner to produce the physically separated zones of substances z_1 , z_2 , etc., along the length of the rod. The thus formed thin layer chromatograph is then placed in a vacuum in close proximity to an ion source 43 of a mass spectrometer. Suitable means such as the mechanical advancing mechanism 44 are provided extending within the vacuum enclosure for selectively advancing the glass rod 39 past inlet 43a to the ion source, so as to successively position each of the zones of absorbed substances adjacent the inlet 43a. A pair of electrodes 46 and 47 are provided adjacent the glass rod and on an opposite side thereof with respect to the inlet 43a. The electrodes 46 and 47 are in electrical contact with the metal spiral 41 on the glass rod 39. As mentioned before, the metal spiral 41 is preferably formed of a metal having a relatively high resistance so as to function as a resistance heater. The electrodes 46 and 47 are connected to terminals 48 and 49 to which an electrical voltage source can be connected for passing an electrical current through that portion of the metal spiral 41 between electrodes 46 and 47. In this

manner heat is produced to selectively evaporate each of the zones of absorbed substances z_1, z_2 , etc., from the thin layer chromatograph through inlet 43a into the ion source of the mass spectrometer. As each of the zones is evaporated into the mass spectrometer ion source, a mass spectrograph is run in the conventional manner.

In FIG. 5 there is shown another embodiment of a thin layer chromatograph similar to the one shown in FIG. 4 and which can be utilized in the same configuration as the chromatograph shown in FIG. 4. In the embodiment of FIG. 5 a rectangular support member 51, which may be glass for example, has a plurality of metal strips 52 formed thereon. Thereafter, a suitable chromatograph medium 53, such as silica-gel, for example, is formed in a strip down the center of member 51 in a direction perpendicular to the strips 52. A thin layer chromatograph is then run in the conventional manner by depositing a sample i of the mixture to be analyzed at one end of medium 53. Thereafter the sample i is chromatographed in the conventional manner using a suitable mobile phase forming a plurality of physically separated zones z_1, z_2, z_3, z_4 and z_5 along the length of the medium 53. The thus formed thin layer chromatograph is placed in a vacuum enclosure in close proximity to the ion source of a mass spectrometer. A pair of electrodes 54 and 55 are situated so as to contact those of the metal strips 52 which are underneath the particular zone z_1, z_2 , etc., which is in close proximity to the ion source of the mass spectrometer. The electrodes are connected to terminals 56 and 57 to which an electric voltage source then passes an electric current through the metal strips 52 which are contacted by the electrodes 54 and 55. The metal strips 52 are preferably formed of a metal having a relatively high resistance so that this current generates sufficient heat to evaporate the substance z_1, z_2 , etc., into the ion source of the mass spectrometer. As was the case in the embodiment of FIG. 4, mechanical means may be provided for advancing the thin layer chromatograph step-by-step so as to successively position each of the zones in close proximity to the ion source. As each of the zones is thus positioned and evaporated, a mass spectrograph is run in the conventional manner.

Thus what has been described is a method for analyzing mixtures of substances in which a thin layer chromatograph is combined with a mass spectrometer. In accordance with the invention the thin layer chromatograph itself is placed in a vacuum in close proximity to the ion source of a mass spectrometer. Heat is selectively applied to the substances of the chromatograph to directly evaporate them into the ion source of the mass spectrometer. Although the method has been described with respect to a few illustrative examples, obviously modifications may be made to the specific embodiments disclosed herein without departing from the true spirit and scope of the invention. For example, it

is contemplated that any size chromatographic apparatus such as a fine quartz fiber or tubular fiber may replace the "glass rod", "tube", or "plates" specifically referred to in the above description. Further, materials other than glass, such as ceramic, silica, metal or other inert low vapor pressure substrates may be used.

What is claimed is:

1. A method for analyzing a mixture of chemical substances comprising the steps of forming a thin layer chromatograph of the mixture whereby differential migration of substances in the mixture forms a succession of absorbed zones of the substances along a chromatograph medium to define the thin layer chromatograph, positioning the thus formed thin layer chromatograph in relatively high vacuum adjacent to and communicating with the ion source of a mass spectrometer, successively heating each of the absorbed zones whereby as each zone is heated the absorbed substance sublimates directly into the ion source, and forming a mass spectrograph of each of the thus subliming substances.

2. A method in accordance with claim 1 wherein selective heating of the absorbed zones is accomplished by selectively directing a beam of light upon each of said absorbed zones.

3. A method in accordance with claim 1 wherein selective heating of the absorbed zones is accomplished by providing an electric resistance heater element adjacent each of the absorbed zones and passing current through the heater element.

4. A method in accordance with claim 1 wherein the thin layer chromatograph is formed on the inside surface of a cylindrical support which is open at both ends with one of its ends adjacent the ion source, and wherein selective heating of each of the absorbed zones is accomplished by passing an electrical resistance heating element down the length of the cylindrical support on its exterior, and including the step of providing a flow of an inert gas into the opposite end of the cylindrical support to carry subliming zones of substances into the ion source.

5. A method in accordance with claim 1 wherein a support member has electrical resistance heating elements formed thereon, with a chromatographic sorptive medium formed on top of a portion of the electrical resistance heating elements and the thin layer chromatograph formed along the sorptive medium, and wherein successive heating of the absorbed zones is accomplished by successively passing an electric current through the resistance heating elements underneath each of the absorbed zones.

6. A method in accordance with claim 5 including mechanical advancing means to successively displace the thin layer chromatograph with respect to the ion source so as to successively position each of the absorbed zones in close proximity to the ion source.

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